A STUDY ON PREVALENCE OF SUPERFICIAL MYCOSES AND ITS ANTI-FUNGAL SUSCEPTIBILITY PATTERN IN DIABETIC PATIENTS IN A TERTIARY CARE HOSPITAL

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CERTIFICATE

This is to certify that this dissertation titled **"A STUDY ON PREVALENCE OF SUPERFICIAL MYCOSES AND ITS ANTIFUNGAL SUSCEPTIBILITY PATTERN IN DIABETIC PATIENTS IN A TERTIARY CARE HOSPITAL"** is a bonafide record of work done by **DR.P.PONNAMMAL,** during the period of her Post graduate study from 2010 to 2013 under guidance and supervision in the Institute of Microbiology, Madras Medical College and Rajiv Gandhi Government General Hospital, Chennai-600003, in partial fulfillment of the requirement for M.D. MICROBIOLOGY degree Examination of The Tamilnadu Dr.M.G.R. Medical University to be held in April 2013.

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DECLARATION

I declare that the dissertation entitled **““A STUDY ON PREVALENCE OF SUPERFICIAL MYCOSES AND ITS ANTIFUNGAL SUSCEPTIBILITY PATTERN IN DIABETIC PATIENTS IN A TERTIARY CARE HOSPITAL”** is submitted by me for the degree of M.D. is the record work carried out by me during the period of October 2011 to September 2012 under the guidance of **Prof.Dr.T.SHEILA DORIS, M.D.** Professor of Microbiology, Institute of Microbiology, Madras Medical College, Chennai. This dissertation is submitted to the Tamilnadu Dr.M.G.R. Medical University, Chennai, in partial fulfillment of the University regulations for the award of degree of M.D., Microbiology (Branch IV) examination to be held in April 2013.

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INTRODUCTION

Diabetes mellitus is a chronic metabolic dysregulation of glucose. It is a world-wide problem of increasing importance. It affects approximately 285 million people and likely to rise to 438 million by 2030. [1,17] .In Asia, where two thirds of the world's diabetic patients live , the type 2 diabetes accounts for 90% of the cases[2].Population based analysis states that the prevalence of diabetes in India was 31.7 million in 2000 and will rise to 79.4 million in 2030.[1]

Cutaneous infection accounts for 20-50% of the skin manifestation among the diabetic patients due to poor glycaemic control [2-4,17].More often these cutaneous signs heightens the suspicion of a physician regarding the diagnosis of the disease [5,6].

The fungal infection is the commonest cutaneous infection in diabetic patients. Moreover the prevalence of superficial mycoses namely onychomycosis and tinea pedis is 75% in diabetic patients especially in type 2. [7-9]

The relative occurrence of the etiological agents of these superficial mycotic infections varies among countries[10]. Hence this study was conducted to know the prevalence and the causative agents in our region.

Diabetes is often associated with various serious complications, in particular diabetic foot ulcers .This causes considerable morbidity, disability and also the leading cause for foot amputations[94] and hospitalization[1,11,30].

Onychomycosis and tinea pedis are the main predisposing factors for the development of these dreadful complications since it disrupts the skin integrity and allows the entry of secondary pathogens (bacteria). [1,12]. Further the condition is associated with secondary immunodeficiency [12], Peripheral neuropathy, arterial insuffiency, retinopathy , minor trauma and obesity [1,13] which also acts as major risk factors.

Hence early detection of superficial mycoses especially tinea pedis and onychomycosis of toe nails which are not commonly noticed by the patient and timely, adequate dosage of antifungal agents with good glycaemic control can save the limb.

Skin and nail infections are more common in diabetes [14]. The commonest causative agent are the dermatophytes (57%) [7] which constitute a group of superficial fungal infection of keratinized tissue viz the epidermis, hair and nail [15]. Although dermatophyte infections are not more common in the diabetic population when compared to normal populations, they are of special concern because of the secondary complications.[16].

Candida species constitute 28% of cutaneous infection [7] and its incidence is high in diabetes because of the decrease in the β globulin, an anti candidial factor [17]. Infections with candida species, correlates well with the increased blood glucose levels and thus it helps in identifying any undiagnosed diabetes mellitus cases [Orman 2001] [17].

Several candida species are involved in human infections. Among these, Candida albicans is the most common species. However during the past

decade, there is an emergence of non albicans candida species especially among the diabetic patients due to immunosuppression [26].

Most common non-albicans isolate were C.parapsilosis, C.glabratra and C.tropicalis.Candida albicans and non albicans species though related, they differ in their epidemiology, virulence factor and antifungal susceptibility pattern. The inappropriate use of antifungal drugs and easy availability of the counter drugs in countries worldwide has predisposed to the development of resistance to antifungal drugs.

Hence isolation and anti-fungal susceptibility testing of these isolates will help to choose the appropriate sensitive agents thereby clear the infection and prevents secondary complications.

Apart from these agents, uncommon non dermatophyte moulds can also cause 2-7% of the superficial fungal infections in diabetic patients. These includes Aspergillus spp,Fusarium spp,Scytalidium spp,Acremonium spp and Rhizopus spp [57]

Thus diabetic patients due to their immunosuppressive states are prone for frequent fungal infections of the skin and nails. Hence their early diagnosis, isolation of the causative agents[73] can prevent major complicatons. Antifungal susceptibility testing in these patients can help to identify the emergence of any resistance patterns.

REVIEW OF LITERATURE

HISTORY

Mycology ,the study of fungi came into existence before bacteriology, in 1677 when Hooks studied the yellow spots on the leaves of Demask rose with the help of a magnifying lens and found the filamentous organism.Subsequent studies were carried out by Malphigi (1686);Mitchelli (1729) and Linnaeus (1752).[1,18]

The lesion produced tends to creep in a circular or ring form. For this reason, the Greeks named the disease *herpes*-a term which still persists[19] and the Romans named the disease as 'Tinea',which means, a small insect larva.This name is still being used to clinically describe the lesion.[19] The English word, "Ringworm", is a combination of meanings in Greek and Latin

The chronic inflammatory diseases in scalp with loss of hair and formation of folded, crusted scutula have been recognised in central Europe and the Mediterrean area since classical times.

In 1834, Remak examined materials from favus and noted the presence of filaments resembling mould. He tried to reproduce the disease by rubbing the organism on his skin, but failed ..Later Schoenlein in 1839 described the filaments as being those of moulds and concluded that favus was a disease of plants.[18]

In 1841 David Gruby, published a paper in which he described the isolation of fungus of favus on potato slices and production of the disease by

inoculating onto normal skin. Thus he was the first to establish the role of microorganism in the causation of favus. In addition he described the dermatophyte, *Microsporum audouinii* from tinea capitis and recognised the endothrix form of *Trichophytons* [20]

Malmsten defined the genus *Trichophyton* and *T.tonsurans. T.mentagrophytes* was defined in 1847 by Charles Robin who was the first to discuss the topical therapy for dermatophyte infections.

Domenico Majocchi was the first to describe the variant of tinea corporis popularly called as Majocchi's granuloma,a dermal and subcutaneous tissue infection caused by dermatophytes and he named it as 'Granuloma Tricofitico" in 1883.[20]

By 1890, Sabouraud, published his systematic and scientific studies on dermatophytoses. His book *Les Teignes* is considered a classic in medical literature where he classified the dermatophyte into three genera,*Microsporum,Trichophyton,*and *Epidermophyton* along with the genus *Achorion* based on clinical rather than on botanical observation[20]

In the 1920's Hopkins and Benham began their scientific study in medical mycology and Rhoda Benham was called as the founder of modern medical mycology. The laboratory at Columbia University was one of the first to study clinical mycology systematically.

In 1925 Baltimore a Physicist, and Robert W.Wood invented Wood's lamp which was used for detection of fungal infection of hair.[20]

In 1934, Chester Emmons redefined the dermatophytes according to the botanical rules of nomenclature and taxonomy.Lucille George identified several organisms based on physiological characters and nutritional requirements into sixteen species of dermatophytes.[20]

In her review Ajello, described the species in *Epidermophyton* as two, *Microsporum* as 16 and *Trichophyton* as 21. C.W.Dode, 1935 published the association of locally endemic dermatophytes with a particular population groups. He also detailed about the immunology, pathology and distribution of fungal diseases.

Weitzman et al restudied the teleomorph state of all dermatophytes with sexual phase. Ajello, Dawson and Gentles, in 1959 discovered the teleomorphs of *Trichophyton* using hair bait technique of Vanbreuseghan(1952)[21].Griffin and Stockdale in 1960s independently obtained teleomorphs of *Microsporum gypseum* complex, there by proved Nannizzia's original observation of the sexual stage. [20]

Williams in 1958 described the first cure in human patient with griseofulvin.In 1969, Taplin and co-workers developed DTM to isolate and differentiate dermatophyte from other fungal and bacterial contaminant in cutaneous lesions. [22]

Blank and co-workers established the dosage and treatment schedules which were widely accepted as the treatment of choice for all forms of dermatophytoses.Several topical preparation have also been introduced of which tolnaftate has gained wide popularity.

More recently imidazoles-clotrimazole, ketoconazole, miconazole and econazole have been used as topical agents. Treatment failures and relapses occur with all the presently available antifungal drugs. Hence there is a need for better therapeutic agents.

MYCOLOGY

Most of the fungal infections of the skin, hair and nail are caused by dermatophytes, Candida and Pityriasis versicolor .Other less frequent infections of skin and hair include tinea nigra and piedra.In addition there are number of non dermatophyte moulds that can cause nail infections (onychomycosis).

Superficial fungal infections, such as dermatophytosis and onychomycosis have an important problem in diabetic patients due to immunosuppression [23].

ETIOLOGY

The organism that cause dermatophytosis (keratinolytic fungi) are moulds belonging to the genera *Trichophyton, Microsporum* and *Epidermophyton.*[23] . They all belong to the,

Family-Arthrodermataceae

Order-Onygenales

Class-Plectomycetes

Phylum-Ascomycota

The dermatophytes are hyaline septate moulds with more than hundred species. Forty species are considered valid and less than half of these are associated with human diseases. These are divided into three main anamorphic genera depending on their morphological characteristics [20] as,

Trichophyton 24 species

Microsporum 16 species

Epidermophyton 2 species.

The two perfect genera corresponded closely to the imperfect genera ie, all *Microsporum* species with perfect stages belong to the genus *Nannizzia* and all *Trichophyton* species to the genus *Arthroderma.*

Antigenically and physiologically,the dermatophytes are closely related but few species shows nutritional differences which have been useful in separating similar species.[19]

The *Trichophyton* species usually infects skin, hair and nails. *Microsporum* species infect skin, hair and not the nails and *Epidermophyton* species infect skin as well as nails but not the hair[20](Sherne et al 1993).

These group of organisms are homogenous not only in appearance but also in physiology, taxonomy, antigenicity, growth requirements and infectivity. The ability of these microorganisms to invade and parasitize the cornified tissues are closely associated and is dependent upon the utilization of keratin which is a highly insoluble scleroprotein [19]

ECOLOGY

The dermatophytes can be classified into three ecologic groups depending on their habitat (natural reservoir) as,

Geophilic species- Soil

Zoophilic species- Animals

Anthropophilic species- Humans

ANTHROPOPHILC SPECIES

Anthropophilic species, most commonly causes human infection and has evolved from zoophilic species.[21,22].These are highly contagious since the arthroconidia and chlamydoconidia shed in the environment ,along with the desquamated epithelium and hairs can survive longer (Mc Pherson 1957, Kwong Chung 1992).[21].

Fomites also play a role and infection can be acquired through aerosolisation of arthroconidia into the air,e.g *M.audouinii* –Ectothrix tinea capitis and *T.tonsurans* –Endothrix tinea capitis in children (Houchins and Pugliase 1991) and in adults ,tinea corporis,tinea manuum ,tinea unguium (Summerbell ,Weitzman 1995).

T.tonsurans can cause nosocomial infection through aerosolisation. [21]. *T.rubrum,T.mentagrophytes* and *E.floccosum* can cause tinea cruris,tinea pedis and tinea unguium but varies with the geographical locations

Other anthropophilic dermatophyte species like *T.violaceum* and *T.schoenleinii* can also be transmitted via fomites (Kwong Chung

1992).*T.concentricum* can be transmitted after birth to the neonate from the mother either directly (Rippon 1988) or through fomites.(De vroey 1985)[21].

Tinea pedis is acquired by direct exposure to the fungal spores commonly seen in shower heads, swimming pool and locker rooms (Gentles 1957). Epidemiological evidence as analysed by Gentles et al (1957) lstrongly documented a 3-fold increase in the incidence of tinea pedis cases among coal miners using the communal showers [21].

GEOPHILIC SPECIES

The soil contains many keratinophilic fungi closely related to the dermatophyte genera and are secondarily transmitted by animals to human [21,22]. These includes *M.gypseum, M.fulvum, T.ajelloi* and *T.terrestre*.Most of these organism are rarely isolated from human infections.

ZOOPHILIC SPECIES

Zoophilc species evolved from geophilic species and can cause human infections [21,22] Infection caused by the zoophilic dermatophyte,*M.canis* can involve the domestic animals as the principal carriers (,DeVroey 1985, McGinnis 1985, Kwong-Chung 1992).

T.verrucosum and *T.mentagrophytes var.mentagrophytes* are mostly acquired from cattle.In Tinea corporis, tinea capitis and tinea barbae ,fomites play a role in the transmission of *T.mentagrophytes var quinckeanum* (Georg 1960). *T.mentogrophytes var mentogrophytes* (granular variety) is carried by rodents and can transmit to laboratory workers (Georg 1960,Sewell 1995).[21]

Infections acquired from zoophilic species are inflammatory hence resolve spontaneously than infection caused by anthropophilic species which are non-inflammatory (Rippon1988,KwongChung 1992)[21]. Other zoophilic agents are *M.gallinae,T.equinum* and *M.nanum.*

EPIDEMIOLOGY:[21P223-33]

Dermatophytes affect 20-25% of the world's population and the incidence continues to rise [24, 25]. The distribution of superficial mycoses varies among countries. Hence investigators are keen in identifying the etiology, distribution, pathology and the treatment strategy,

Dermatophytes survive at 25-28^{0}C .Hence in tropical countries, the humid warm conditions on human skin supports its growth. [26]. Dermatophytes are geographically restricted and endemic only in particular parts of the world [19].

Skorepova underlined that the frequency of skin mycoses in diabetes mellitus is not significantly higher than that in general population. But Garcia-Humbria et al demonstrated prevalence of superficial mycoses as 75% in diabetes in contrast to 65% in non-diabetic healthy individuals. [27]

In Indians, occlusion of the infected sites appears to increase the susceptibility to chronic infection by increasing the hydration and the emission of CO_2 which favours the growth of dermatophytes (King et al1978). Within a country, the anthropophilic and zoophilic species are not static and can change as a result of population shifts [21].

In North India anthropophilic species are the commonest pathogens causing tinea capitis. In a study on 153 consecutive patients with tinea capitis,90% of the patients were aged less than 15years; and the causative strains were.*T.violaceum* (38%),*M.audouinii* (34%),*T.schoenleinii* (10%) and *T.tonsurans* (10%).[26]

In South India, *T.violaceum* is the common strain causing tinea capitis .A study in 1978, proved the occurrence of tinea capitis among boys aged 10-17years due to the unhygienic mass scalp shaving rituals [26].

The commonest causative agents of tinea pedis are *T.rubrum* and *T.mentagrophytes* and *E.floccosum*.

Immunosuppressive states especially diabetes mellitus and oldage, are predisposed to chronic infections [12,28]**.** An inherited pattern of infection with *T.concentricum* is also noted (Serjeantson and Lawrence 1977).[21]

AGE DISTRIBUTION

Scalp infections are common in children but can rarely occur after puberty. But this infection causes scarring alopecia in adults. The reason for the preponderance of the disease in children is due to the presence of medium chain fatty acids in sebum which inhibits the growth of dermatophytes in post pubertal individuals.[29]

In constrast, tinea pedis is usually seen in adolescents or young adults. Foot infection occasionally occur in young children,but with concomitant skin infection.[29]. The prevalence of onychomycosis with tinea pedis is seen more among diabetic patients [7].

Many of the 40 species are distributed world-wide but the remaining strains are confined to specific regions. Hence the study on their ecology and epidemiology are essential to control the infections. [21].

IMMUNOLOGY:[21]

Dermatophyes initially colonize the stratum corneum. The infection and the inflammatory reaction depend on the causative species and the host immunity. Infections with anthropophilic species elicits less inflammatory response when compared to zoophilic and geophilic species

HOST RESPONSE

- Innate immunity (Nonspecific)
- Acquired immunity (Specific)

INNATE IMMUNITY

The epidermis and nails synthesize a natural peptide which possess antimycotic activities. These peptides are glycated in uncontrolled diabetic patients hence they lose their protective function.[30]. .

Dermatophyte antigens act as chemokines for the leukocytes in inflammatory lesions. These neutrophils kill the pathogen through the oxidative pathway. It also activates the alternate complement pathway.[29]. In diabetes mellitus,due to the glycation of C3 the attachment to the surface of microorganisms is inhibited[27].

In diabetic patients, leucocyte chemotaxis, adherence and phagocytosis are impaired during hyperglycaemia and are prone for chronic dermatophytoses.[17,27].

ACQUIRED IMMUNITY

HMI

The humoral immune response does not appear to help in the elimination of infection and the highest levels of antibodies are often found in patients with chronic dermatophytosis.Nonspecific antibodies are produced which cross react with other dermatophytes and saprophytic fungi.

IgE suppresses the CMI through its histamine secretion and HMI is variable during dermatophytosis.There is no standard antigen available to test its sensitivity and specificity.Matsumoto et al1996).

CMI

CMI is by type IV delayed hypersensitivity reaction mediated by cellular immune system which is important in eliminating the infection from stratum corneum,[31] and thus results in both mycological and clinical cure .(Dahl 1993).

Trichophytin skin test is positive in normal population because of their earlier exposure to dermatophytoses or by cross reactivity to other organisms. (Grossman et al 1975) [20].

Defective T-cell mediated response causes chronic infection with *T.rubrum* and *T.concentricum*.Persistent dermatophytic infection elicits Th2

immune response.[29] Moreover cutaneous T cell function and response to antigen challenge are depressed in diabetic patients.

The dermatophyte species vary among themselves in eliciting immune response,like *T.rubrum* causes chronic or relapsing infection while *T.verrucosum* causes long term resistance to re-infection.[29]

PATHOGENESIS

Dermatophytes colonize and adapt to grow in the living keratinised layer of stratum corneum ,in the nail plate ,nail bed and around hair shafts and thereby develops equilibrium with the host . They produce only less irritation to the specific host.

Dermatophytes slowly develop specialised methods of reproduction and easily disseminates from host to host, by the formation of arthroconidia. [32, 33]. These arthrospores are the vegetative cells with thick cell wall which transfers the infecting agents from the original specific hosts. [33].

The acquisition of infection depends upon the skin surface factors like local CO_2 tension, moisture and unsaturated transferrin. For penetration it needs, zinc containing metalloproteinase.[33] The ratio of epidermis to serum glucose concentration is higher in patients with diabetes mellitus which may favour fungal growth.[27]

Human genetics also play a role in the pathogenicity. Autosomnal dominant trait is seen in some families with onychomycosis and autosomnal recessive trait is seen in tinea imbricata.[34]

Several host mediated factors can also limit dermatophytoses, like Progesterone (Heoprich et al 1994) hence there is an increased incidence of some dermatophytoses in men .Unsaturated fatty acid in sebum also can inhibit the growth of dermatophytes. Thus the production of sebum in adult scalp protects against tinea capitis.[34]

The granular form of *T.mentagrophytes var.mentagrophytes*, a zoonotic infection when infects man, evokes a primary irritant reaction followed by severe inflammatory response which leads to rapid termination of infection.

Moreover these fungal infections of hair produce numerous saprophytic conidia in cultures. On the other hand the anthropophilic species, *T.mentagrophytes var.interdigitale* elicits only little inflammatory reaction and causes chronic infections and also produces only few saprophytic conidia in cultures.[19]

On glabrous skin, dermatophytes produce the classical ring worm pattern with centrifugal spreading. Most of the dematophytosis resolve apparently but some may persists as carriers.[20].

VIRULENCE FACTOR:[19,21,22,35,36]

- Dermatophytes produce a number of keratinolytic proteinases that function best at acidic pH and these have been recognized as an important virulence factors.[37].

- The molecular structure of keratin varies from species to species, hence different keratinases have been evolved with relative specificity. [19].

- Another factor which enhance the pathogenicity is the mannan produced by its cell wall (Blak et al, 1991) [34] .which suppresses the CMI.

- Dermatophytes produce catalase enzyme.

- Dermatophyte also produce enzymes like chitinase or proteinase to derive nutrition from the epidermal structures.

CHRONIC DERMATOPHYTIC INFECTION:[21,35]

Some of the patients are prone for chronic or recurrent infection due to the following reasons,

- Skin provides an environment favourable for fungal growth and persistence.

- Immunosuppression (diabetes) is another cause of increased vulnerability to fungal infection.

- Atopic or those who lack CMI to certain dermatophytes.

- Recurrence due to under treatment or short term therapy.

- Resistant to antifungal drugs.

CLINICAL FEATURES

The clinical feature is a combination of direct tissue damage and the host immunity. Infection is also enhanced in damaged skin like macerated skin as seen in tinea cruris and tinea pedis.[34].

Clinical signs vary with the host and species of the mould. The lesion is localised in a circular pattern with features of erythema, scaling and pruritis. It spread outwards with healing at the center. [38]

TINEA CAPITIS

It is common among children. Anthropophilic species causes endemic infections and zoophilic sporadic [29]. It can be caused by sharing of contaminated comb, clothing [37]. The predominant cause of tinea capitis is *Trichophyton* species particularly *T.tonsurans*.

Anthropophilic species are the predominant agents in India.*T.violaceum* and *M.audouinii* are the causative agents in North India and *T.violaceum* in South India[24]

Scaling may be present on the scalp with minimal Inflammation but marked with *M.canis*. Hairs infected with these agents fluoresce green when exposed to Wood's lamp. [33].Clinically classified as,[26].

- Non inflammatory- *M.audouinii,M.ferrugineum*
- Inflammatory-*M.canis,M.gypseum*
- Black dot type- *T.violaceum, T.tonsurans*
- *Trichophyton* species may cause Ectothrix or Endothrix infections.

In endothrix infections,hyphae form arthrospores within hair shaft and in ectothrix outside the hair shaft.[29].

Trichophyton infection of scalp ranges from scaling folliculitis to kerion formation. *T.mentagrophytes* and *T.tonsurans* commonly cause kerion. *T.schoenleinii* infection causes pustular follicles called as favus which forms crust and scutula along the hair shaft.

Mankodi and Kanvindae (1969) showed that tinea capitis accounts for 10% of all dermatophytosis. The age group being below 13yrs with male predominance and 75% of the isolates were *T.violaceum* [39].

Kamalam and Thambiah (1979) when studied the prevalence of tinea capitis in two schools found ,inflammatory lesion in one school children caused by *T.violaceum*(6.2%) and non-inflammatory lesion in the second school children but *T.violeceum* was isolated in 59.75% cases.Hence concluded that the clinical response depends on the species rather than the host immunity [40]

TINEA BARBAE

T.verrucosum and *T.mentagrophytes var.mentagrophytes* were the principal causative agents. The characteristic appearance is of a localised, highly inflammatory pustular follicules. Some infections are less severe with circular, erythematous, scaly lesions [33]

TINEA FACIEI

The causative species are *T.rubrum* and *T.mentagrophytes var mentagrophytes* , *T.tonsurans* and *M.canis*. The typical annular lesions are erythematous pruritic,but scaling is often absent. The lesions are often exacerbated by sun exposure [29].

TINEA CORPORIS

It is the most common infection [39, 41].The clinical features depend on the species of the infective organism. The disease often follows contact with infected animals, but occasional cases are with geophilic species (*M.canis and T. verrucosum*).

Infections with anthropophilic species can be secondary from tinea pedis and are commonly seen as a nodule on the lower part of the legs with the overlying skin appearing dry,red and scaly.[29]

The characteristic lesion is an annular scaly plaque with a raised erythematous border and central clearing. In their most florid form, the lesions can become indurated and pustular which is very common with zoophilic species. Sites commonly involved are the trunk and legs.

Mankodi and Kanvindae (1969) found that tinea corporis was the commomest dermatophytosis (52,8%) in Ahamedabad and in Bombay (54%) by Mehta Deodhar chaphekar (1977).

TINEA CRURIS:

Infection of the groin and the perianal region.Perineal regions is more often affected in men. The predominant causes are the anthropophilic species ,*Trichophyton rubrum* and *Epidermophyton floccosum*. The infection can be from other sites but person to person spread is also not uncommon.

The lesions are erythematous and have raised scaly margins which radiate from the groin down the inner border of the thigh. Patients often complaints of intense pruritis [33]

Panda, Mohanty and Nanda (1967) from Burla reported the commonest dermatophytoses as tinea cruris accounting for 54.65% with 98.5% cases noted in males and the commonest isolate was *T.rubrum*(78.4%).

TINEA IMBRICATA

A variant of tinea corporis caused by *T. concentricum*.This is a chronic infection characterised by the development of homogenous sheets or concentric rings of scalings that can spread to cover a large parts of the affected person.[33]

TINEA PEDIS

Infection of the feet is most often seen in diabetic patients.Casautive agents being *T.rubrum,T.mentagrophytes var interdigitale* and *E.floccosum*. The most common clinical presentation is interdigital or web space infections which commonly involves between the fourth and fifth toes.

Another common feature associated with *T.rubrum* infection is the hyperkeratosis of the sole, which is presented as dry ,white scaly lesions. This form of the disease is often chronic and resistant to treatment. If there is extensive involvement of the foot, then the term 'moccasin 'or dry type tinea pedis'is often applied.

A third form of tinea pedis, associated with *T.mentagrophytes var.interdigitale*,is the vesicular lesion involving the soles. This acute lesions usually resolves spontaneously. Sometimes exacerbations tend to occur under hot humid conditions. This condition is often associated with hyperhidrosis [33]

Desai and Bhat (1961) reported a higher incidence of tinea pedis among those wear shoes and socks.Baer and Rosenthal (1966) in their experimental study found that trauma played a contributory role.

TINEA MANUUM

It is usually unilateral. Lesions appearing on the dorsal side shows similar appearance to tinea corporis, with distinct border and central clearing. Infection of the palms is more common. This presents as a diffuse scaling hyperkeratosis, with accentuation of the fissuring in the palmar creases.*T.rubrum* is the most common etiological agent.

TINEA UNGUIUM: (ONYCHOMYCOSIS)

Onychomycosis,the fungal infection of nail is the commonest superficial mycosis seen among diabetic patients. Atleast 80% of fungal nail infections are caused by *T.rubrum* and *T.mentagrophytes*.[27,42].Saunte et al revealed 93% of onychomycosis were due to dermatophytes.[27]

The various clinical forms of tinea unguium are,

- Distal and lateral subungual onychomycosis (DLSO)
- White superficial onychomycosis (WSO)
- Proximal subungual onychomycosis (PSO)
- Total dystrophic onychomycosis (TDO)

DLSO is the commonest type**.** This usually begins as a discoloration and thickening of the distal and lateral borders of the nail. Finally it results in destruction of the entire nail plate and separation of the nail from the nail bed.

White superficial onychomycosis is common in toe nails and causes white crumbling lesions involving only the nail surface.This condition is most commonly caused by *T.mentagrophytes Var.interdigitale*.

Proximal subungual disease is a rare presentation involving the finger nails.The nail appears as whitish yellow with periungual inflammation.In total dystrophic onychomycosis, there is complete loss of nail plate.

Onychomycosis or tinea pedis in a diabetic patient should be monitored and treated earlier, as it can disrupt the skin integrity .In untreated cases, these can act as a reservoir and can further favours secondary bacterial infections.

Ravinder Kaur et al (2008) in clinicomycological evaluation of onychomycosis isolated *T.rubrum* (46.67%),*T.mentagrophytes* (20%) ,*T.tonsurans*(4%) and reported that the infection was more common in males in the age groups of 21-30 and 61-70 years. [42]

Dermatophytoses in toe web space leads to inflammation and fissuring. In diabetic patients these fissures can result in serious complications like paronychia and also act as a portal of entry for bacteria.

LAB DIAGNOSIS

The diagnosis of dermatophytosis is based on combination of clinical observations supplemented by laboratory investigation. [20]

COLLECTION OF SPECIMEN

After decontaminating the affected area, the specimens of infected skin, hair and nail are collected in a dry sterile container. [43]

The laboratory diagnosis depends on [20]

- ❖ Demonstration of the causative pathogen in tissue by microscopy.
- ❖ Isolation of fungus in culture
- ❖ Serological tests.

DIRECT MICROSCOPY

It is not a sensitive test for detecting dermatophytoses but it is more rapid, simple method of determining the etiology of an infection when the test is positive [20,43]. Moreover it is helpful in determining whether the organism recovered later in culture is a contaminant or a pathogen and also to select further specific culture medias and tests.[34]

The clinical material should be examined by suspending a portion of the sample in a clearing agent, KOH -10% used for skin and hair but 20% for nails.

In KOH mount, the fungus is seen as branching hyaline mycelia,which frequently show arthrospore production. The demonstration of fungus in nails may be difficult and may be possible only after keeping clippings in KOH for overnight. For all types of clinical specimens, fungal hyphae must be differentiated from other artefacts. [20]

MODIFICATION OF KOH MOUNT:[21P235]

- ❖ KOH with dyes or blue black ink
- ❖ Addition of DMSO (36%) to KOH (20%). Others being dimethylacetamide and dimethylformamide.

- ❖ Glycerine (5-10%) to 10-25% KOH.
- ❖ 10% Sodium disulphide solution.

OTHER SPECIAL STAINS

- ❖ Calcoflour white stain
- ❖ Periodic acid Schiff stain (PAS)
- ❖ Gomori's methenamine silver stain (GMS)
- ❖ Immunofluorescence stain

CULTURE

The clinical specimen should be inoculated on fungal culture media irrespective of their findings in direct microscopic examination [20].

Cultures of dermatophytes require media containing antibiotics. Because specimen from cutaneous sites almost always contain the normal bacterial flora of the skin, hair and nails in addition to saprophytic fungi from the environment.

MEDIA

- ❖ Emmon's modified SDA with antibacterial agents such as chloramphenicol and or gentamicin and cycloheximide to reduce growth of saprophytic fungi.
- ❖ SDA with chloramphenicol should always be included in any regimen for culture.[43]

The inoculated cultures are incubated at 25^0 C, 30^0 C and 37^0 C. The growth is relatively slow and takes 10 days to three weeks.[20] *T.verrucosum* and some strains of *T.tonsurans* grow only at 37^0 C.

At the onset of sporulation and pigment production growth is examined by LPCB mount.The cultures are examined three times weekly for four weeks and appropriately sub cultured onto SDA.

DERMATOPHYTE TEST MEDIUM: [REBELL&TAPLIN 1974]

DTM is used for presumptive identification of dermatophytes [43]. All samples of dermatomycosis can also be inoculated onto the DTM and incubated at 25^0C.This selective primary medium is helpful in isolating pathogenic species from cutaneous specimen. Thus DTM can be used to isolate and distinguish dermatophytes from the saprophytic fungus.

PRINCIPLE

Dermatophytes utilize the proteins present in the medium and turns the medium red by raising the pH (alkaline metabolite) indicating their presence, while most other fungi and bacteria utilizes the carbohydrate in the medium and hence no color change or pH occurrs.

PROCEDURE

The sample should be inoculated onto the agar as soon as possible with a sterile forceps and is incubated at 22-25^0C for upto 14 days.The culture should be examined daily for change in color of the medium and evidence of fungal growth for 7-10days and later discarded.

INTERPRETATION

- Red color media with white cotton like growth.-Presumptive identification of dermatophytes.
- Yellow color media with no growth - No dermatophyte in the sample.
- No color change in the medium with white/off white creamy growth-C.albicans.

DISADVANTAGE [21 pg236]

Pigment production cannot be appreciated.It is only a screening media and not a specific media for dermatophytes since non pathogenic species can also change the color on prolonged incubation.

OTHER MEDIA [43]

- DIM-Dermatophyte identification medium
- SDA with yeast extract.
- PDA-potato dextrose agar
- PFA-potato flake agar
- Corn meal agar

pigmentation are better seen and to enhance sporulation

- Trichophyton agars (1-7)
- Inhibitory mould agar
- Littman oxgall agar with antibiotics (Summerbell et al1989)
- Casamino acid/Erythritol/Albumin
- BCP/Casein yeast extract.

COLONY MORPHOLOGY [43]

Colonies with one pigment on the surface of the colony and another pigment other than black on the reverse are likely to be dermatophytes. [43]The dermatophyte isolate can be distinguished from contaminant by their compact growth around the inoculum and by the color of colony.[20]

IDENTIFICATION OF DERMATOPHYTES [43]

Trichophyton species:[Malmsten1845] Macroconidia are sparse (8-86x4-14µm) or absent.

1)T.mentagrophytes:

Macroscopy: T.mentagrohytes-type-I colony (zoophilic) are flat,granular,creamy with yellow to tan or reddish brown reverse.

T.mentagrohytes-type-II colony (anthropophilic) are flat and downy with surface pigment cream to light yellow with white feathery fringes and light yellow reverse.

Microscopy

Type-I: Macroconidia are abundant which are clavate to cigar shape,smooth walled with 3-6cells. Microconidia is also abundant which are globose and unicellular (en thryses) or in clusters (en grappe).

Type-II: Macroconidia are sparse or absent.Microconidia also are sparse which are clavate or pyriform.Nodular hyphae are frequently seen.Both types show branching conidiophores at right angles, arthroconidia chlamydoconidia and spiral hyphae.

2)T.rubrum:

Macroscopy:

Type-I: White downy to fluffy colony.The reverse is yellow to blood red

Type-II: Surface pigment becomes tan,yellow or tinged with red and texture is granular. The reverse pigment may be colorless, tan or yellow to brown but eventually a deep wine red color.

Microscopy:

Type-I:macroconidia are sparse or absent but abundant in type-II colony.These are narrow,cylindrical with blunt distal ends and thin smooth parallel walls showing 3-8 cells.

Microconidia are also abundant in type-II colonies and are clavate or tear drop forms arranged singly or occasionally in clusters. Chlamydoconidia, nodular bodies, pectinate hyphae and racquet hyphae are also seen.

T.tonsurans [44]

Macroscopy: Powdery, heaped with yellow surface.

Microscopy: Abundant microconidia but rare macroconidia.

T.verruccosum [44]

Macroscopy: White waxy heaped growth at 37^0C

Microconidia: Plenty of chlamydoconidia with absent macroconidia.

T.Violaceum[32]

Macroscopy: Slow growing violet or deep port wine colour colony with a yeast like consistency .

Microscopy: Distorted microconidia

Microsporum species: [Gruby 1843]

Macroscopy: The colonies are cottony,velvety or powdery with white to brown pigmentation in M.gypseum and yellow orange pigment in M.canis.

Microscopy:

Macroconidia are abundant (6-160x6-25µm) which are large,rough walled,multicellular,spindle shaped.M.canis forms numerous thick walled,8-15 celled with hooked spiny tip.In M.gypseum ,it is boat shaped with 4-6 cell.M.audouinii produces only thick walled chlamydoconidia.

Microconidia are scanty.

Epidermophyton species [Sabouraud1907]

Macroscopy: Slow growing,powdery,greenish brown with suede like surface.

Microscopy: Macroconidia are smooth (20-60x4-16µm) thin, pear or club shaped with 1-9cells.Microconidia are not produced.

MICROSCOPIC METHODS

Tease mount(LPCB)

Cultures are examined microscopically by examining a portion of the aerial mycelium. The material is placed on a slide in a drop of LPCB.The matted mycelium is gently teased with a pair of teasing needle. A coverslip is placed and the morphology observed under microscope.

Slide culture

In a petridish with V shaped glass rod, a glass slide ,a coverslip were placed and sterilised in hot air oven 1x2 cm SDA block is placed on the slide and the corners are inoculated with the fungal colony.The whole set is incubated at Room Temperature after adding distilled water

Cellophane/Scotch tape preparation: [45pg91]

A 2inch piece of cellophane tape is taken and the sticky side is carefully placed on the colony. The speculating colony stick on to it and then the tape is gently placed with the sticky side down on a slide with LPCB.

All preparations are examined for the presence of hyphae, microconidia, macroconidia,, their size, shape and arrangement and other non-reproductive vegetative hyphae (spiral hyphae, racquet hyphae, nodular hyphae).

PHYSIOLOGICAL PARAMETERS

To identify and speciate the isolates which may show similar morphology especially Trichophyton species,Physiological tests are performed.[45 pg237] They includes,

Nutritional requirement:[21]

Trichophyton agar (1-7) is used to differentiate the Trichophyton species,

Temperature:

Most dermatophytes grow at 25-30^0 C.T.verrucosum grows only at 37^{0}C.

Pigment production:

Most of the T.rubrum produces cherry red colour under the colony but to differentiate slow pigment producing variants of T.rubrum from T.mentagrophytes, PDA and CMA are used to induce pigment production.

Urease production [46]

T.mentagrophytes produces urease and T.rubrum does not,Hence to differentiate it Christensen's urease medium is used(Philpot1967,Clayton & midgley1989).Other urease producing Trichophytons are T.megninii , T.raubitschekii.(variant of T.rubrum) and urease negative species is T.erinacei..[21]

Invitro hair perforation test [46,45pg237]

T.mentagrophytes and M.canis can perforate normal human hair (<5yrs of age) producing wedge shaped tunnels or holes.T.rubrum and M.equinum do not perforate and thus can be differentiated.

Rice grain test [20]

Except for M.audouinii,all other microsporum species grow rapidly on sterile rice grains

Hair bait technique

This is performed to isolate geophilic dermatophytes from soil like M.gypseum.

SEROLOGY [20]

The skin test with trichophytin, which is a crude extract produces delayed type hypersensitivity reaction in most adults.The carbohydrate

portion is related to an immediate response whereas the peptide moiety to immunity.Absence of these reaction leads to chronic dermatophytoses.

Immunodiffussion test are done for the diagnosis of dermatophytoses.[20]

ANIMAL INOCULATION [20]

Animal pathogenicity testing is done on laboratory animals like guinea pig, mice and rabbit .Animals can be infected with geophilic and zoophilic dermatophytes. The area to be inoculated with conidia and hyphae is shaved and scarified. The isolates are inoculated and growth is seen in 7days. The lesion usually resolves by 3-4weeks in most of the cases.

MOLECULAR TECHNIQUES: To assess the relatedness and taxonomic classification of the dermatophytes, Mitochondrial DNA analysis of dermatophytes using BG II; HaeIII and PCR are being done

ANTIFUNGAL SUSCPTIBILITY TESTING

Antifungal susceptibility testing has received much attention with the advent of newer antifungal drugs. But it is not advanced as with bacteriology. It must provide a reliable measure of the relative activity and also correlate with in vivo activity. [47].

AFST is done to ascertain the minimum inhibitory concentration by which the in vivo effectiveness of an antifungal drugs can be assessed. Moreover the development of drug resistance can also be determined. [48]. AFST depends on the following parameters,

- pH of the medium
- Inoculum size of the isolate
- Medium
- Time/Temperature of incubation
- Invitro-invivo correlation

METHODS

- Broth dilution(CLSI M38A)-Macrodilution and Microdilution
- Agar dilution
- Disk diffusion
- E-test

TREATMENT

- Antifungal antibiotics
- Synthetic antifungal drugs
- Miscellaneous drugs.

ANTIFUNGAL ANTIBIOTICS

Griseofulvin is a narrow spectrum antibiotic produced by Penicillium griseofulvum and Khuskia oryzae. It inhibits mitosis by interfering with polymerised microtubule and spindle formation.It is fungistatic agent.

SYNTHETIC ANTIFUNGAL DRUGS

- Thiocarbamate: Topical agents-Tolnaftate

Allyiamine and benzylamine: Selectively inhibits squalene epoxidase which is needed for fungal ergosterol synthesis. Hence it is fungicidal.

Azoles: It inhibits cytochrome P450 dependent C14 demethylation in ergosterol synthesis. This causes accumulation of abnormal sterols and ultimately fungal death.

- Imidazole

- Triazoles:

MISCELLANEOUS DRUG

- Ciclopiroxolamine: Pyridine analogue which inhibits fungal cell wall synthesis and also inhibit metal dependent enzyme.used as 1% cream.

- Whitfield's ointment: It is a mixture ofbenzoic acid (fungistatic) and salicylic acid(keratolytic) in the ratioof 2:1.it is used for tinea pedis.

- Castellani paint-1.5% carbol fuschin.

- Undecylenic acid: it is used as soap and foam for tinea pedis.

- Haloprogin:used as 1% cream

- Triacetin:used as 25% cream with cetylpyridinium and chloroxylenol.

PREVENTION [21]

Prevention and control depends on the site of lesion,causative species and its source . For scalp infections,all the contacts are screened by wood's lamp examination for fluorescent hair (Microsporum species) including pet animals. In case of non-fluorescent tinea capitis (T.tonsurans, T.violaceum), the scalp is carefully examined for the presence of spotty alopecia and scaly lesions. The suspected lesion should be cultured regularly.

Tinea corporis and tinea cruris can be transmitted with contaminated clothing, bedding, towel hence washed and disinfected regularly. Avoidance of use of tight fitting, non absorbent cloth and prolonged exposure to wet cloth and weight reduction can prevent the occurrence of tinea cruris.[34pg374]

To prevent the occurrence of tinea pedis /onychomycosis, protective footwear can be worn when using public facilities. To prevent recurrence, measures should be taken to reduce foot moisture, like drying foot after baths and applying antifungal powder.Richardson, Elewski (2000) et al has stated that treatment of tinea pedis can prevent tinea manuum.[34]

For zoophilic infections, the source must be traced and treated. Summerbell and Weitzman (1995) detailed the preventive measures like good sanitation and use of fungicidal sprays.

VACCINE

A live vaccine (LTF130) against T.verrucosum was introduced for cattles in the former Soviet union (Segal 1989) [21].

CANDIDA

HISTORY

Hippocrates *Epidemics* described the candidial infection and candidiasis in two patients in the fourth century B.C.The French word for this condition was 'Le Muguet' meaning 'lilly of the valley'. The first case of oral thrush in modern medicine were made by Rosen von Rosenstein (1771) and later by Underwood (1784)

Candida albicans were variedly named as Oddium albicans (Robin-1853); Syringospora robinii(Quinquad-1868);Saccharomyces albicans(Reess-1875). Grawitz (1877) published the various morphological forms of *candida albicans*

Zopf in 1890 named the fungus Monilia albicans.Dubendorfer (1904) described onychomycosis.Aldo castellani (1912) was the first to suggest the possibility of candida species other than albicans, namely *C.krusei* and *C.tropicalis*.

In Eighth botanical congress (1954) the generic name *Candida* was finally accepted. In 1978 *Torulopsis* was merged into the *candida* genus and named as *C.glabrata*. Research towards *Candida* species were started actively after the advent of antibacterial agents and their indiscriminate usage and also the emergence of AIDS pandemic.

MYCOLOGY

Candida albicans was considered to be the commonest species but frequency of non albicans candida is on the rise.[33] In *Candida species* there are 163 anamorphic species with telomorphs in 13 genera.[20]

PHYLUM-Fungi imperfecti

ORDER-Moniliales

FAMILY –Cryptococcaceae.

Candida posses β glucans in their cell wall and do not produce starch or carotenoid pigments.Serotypes in *Candida albicans*-A and B based on on their difference in mannan component of cell wall.

STRUCTURE

Candida is a small (4-6µm), oval, thin walled yeast like fungi which reproduces by a process of budding. They produce blastospores and pseudohyphae.[34] The cell wall is composed of phosphorylated mannans, glucans and smaller amount of chitin on which the polysaccharide and proteins are intimately bounded.

The difference among *candida* species is in the phospho glycopeptide oligomers and polymers. The cell wall contains active protein, enolase,N-acetyl glucose aminidase,ubiquitin like epitope hsp 70.

Candida is an eukaryotes with a nucleus containing RNA rich single nucleolus and double layered nuclear membrane. The cytoplasmic membrane contains ergosterol, Cytoplasm contains mitochondria, vacuoles, vesicles, endoplasmic reticulum, microtubules, ribosomes and glycogen crystals but Golgi apparatus is absent.

EPIDEMIOLOGY

Candidiasis has no geographical limitation as it occurs in patients who are predisposed to an overgrowth of their endogenous flora.[49].One exception is *C.viswanathii* which is reported only in India(Davis 1986)[21]

Candida is commonly found on skin, GIT,oropharynx and female genital tract.[22].and can be isolated from various sites .There are many factors which predispose to superficial and deep seated candidiasis which can alter the balance of normal microbial flora of the body or lower the host resistance.

Candida species causes infections in all age groups but more commonly in new borns and elderly people. Other predisposing factors includes underlying systemic disease such as diabetes, immunosuppression by disease (AIDS) or medication, antibiotic therapy ,IV drug abusers, haematological malignancy, indwelling urinary catheters.[50] and disrupted epithelial barrier.Warm moist environment favour the growth of candida.[34]

Apart from *Candida albicans* which is the common etiological organism, there is an increasing incidence of non albicans candida namely *C.tropicalis, C.krusei, C.glabrata* and *C.parapsilosis*[1,20] especially among diabetes. In addition, other factors like dimorphic parameter,specific genetic susceptibility have also contributed to the increased prevalence of non albicans candida.

Introduction of various anti mycotics and their indiscriminate usage has resulted in the selection of specific species that are inherently less susceptible to the specific drugs, like *C.glabrata and C.krusei* intrinsically resistant to fluconazole.

VIRULENCE FACTOR [51pg423-30]

- Toxins- glycoprotein extracts of candida cell wall

- Complement receptors- *Candida albicans* has the ability to bind complement derived opsonins through iC3b. (Calderone and Braun1991). As a result they possess the capacity to produce bio films. Hence show poor response to antimycotic drugs. [51,34].

- Chronic hyperglycaemia induces the expression of C3 and this kind of molecular mimicry contributes to easy colonization and infection. [27]

- Adhesion [21]- *Candida* adhere to exfoliated human epithelial and mucosal cells through its mannan (Douglas 1987) and chitin(Segal and Sandovsky-Losica1995,1996). The counterpart on host cell is fucosyl-glucosamine, fibronectin or arginine-glycine-asparagine (Hostetter 1994)[47]. GT formation also helps in adhesion. Adhesion is achieved through nonspecific (electrostatic charge, van der walls force) and specific mechanism (ligand receptor interactions)[52]. Level of adherence correlates well with the pathogenicity. (Samaraanayake et al 1994).

- Phenotype switching-Morphological colony changes from smooth to rough; white to opaque.[53]. Thus it can adapt to various anatomic sites,commensalim to pathogenicity and evade immune defence.

- Yeast-hyphal morphogenetic transformation facilitate penetration and also assist in evading the host defence system.[34] (Shephard,Poulter andSullivan 1985)

- ❖ Enzyme production.-secretory aspartyl proteases (SAP)-tissue penetration and invasion.[21,34] through degradation of keratin and collagen.

- ❖ Phospholipase (ogawa et al 1992) [34] at hyphal tip aids in greater invasiveness.Moreover these hyphal form are larger hence easily phagocytosed.(Diamond and Krzesicki 1978)

IMMUNOLOGY [21]

Both humoral mediated and cell mediated immunity play a vital role.CMI play the major role in mucocutaneous candidiasis. Hence immunocompromised individual are at increased risk of acquiring superficial mycosis.The cells involved are CD4+Th1 cell, which activates macrophages which is a candidacidal, through IFN-ɤ, IL, GM-CSF

Activated immune cells also releases free oxygen radicals and cationic proteins which has antifungal effect. Polymorphonuclear cells can phagocytose and kill candida species through their primary granular enzyme, myeloperoxidase anddefensins (Domer and Carrow 1989;Greenfeld 1992)

Phagocytosis and killing of candida species are also performed by complement, antibodies and cytokines like IFN-ɤ, TNF. Complements act on CR2, CR3 present on C.albicans. Hence diverse cells are involved in the defence mechanism against candidiasis

Immunomodulatory effects of fungal determinants reduces the activity of host's defence system.[34] (Cassone 1989,Domer et al 1992)Hyperglycemia promotes yeast adhesion and diminishes its phagocytosis[51]

PATHOLOGY

Superficial Candidiasis is an endogenous infection. The shift results due to a number of influences,of which host factors appear to be the most important.[26] Local tissue damage associated with immunosuppressive state is the critical factor in the pathogenesis of cutaneous candidiasis in diabetes[54]

Candida being normal commensal, the predisposing factors like diabetes mellitus can impair the immune response to these organism and this imbalance favours mycobiota,which later damages the integrity of the integuments.

CLINICAL FEATURES

Candidal infection (moniliasis) is an early sign of an undiagnosed diabetes.They can present with, intertrigo (axillary,under the breast,groin, inguinal, web space), [Hay1999], paronychia, onychomycosis and glossitis.[17]

SUPERFICIAL CANDIDIASIS

Cutaneous candidiasis is a less common disease causing superficial mycosis than dermatophytes [33] This yeast-like fungus presents as an itchy rash which are surrounded by tiny blisters.[33]

Macroscopically these lesions are characterised by the formation of a greyish plaque, surrounded by oedema. A special feature of these lesion is the formation of warty growth which results from hyperkeratosis and epithelial hyperplasia[34] .

The lesion is influenced by interaction of three factors, namely the site of infection, the pathogenicity of the infecting organism and the competence of host immune response.[34]

Infection of the skin between the fingers or toes can also occur. It is often uncomfortable and may be painful. Infection of webs of the toes mimics tinea pedis and many cases do occur in conjunction with this form of dermatophytosis.

WEB SPACE INFECTION (Erosio interdigitalis blastmycetica)[34]

Most common between the third and fourth finger andfourth and fifth toe due to trapping of moisture. Clinically the skin appears macerated with scaling and fissures.

NAIL INFECTION

Less than10% of the infections are due to non dermatophyte organism and of which 50% is Candida and it is common in immunocompromised. Dogra et al found 48% of nail infection in diabetes were due to yeast like fungi.[27]

a)*Paronychia*

Infection of the nail folds is characterised by inflammation and painful, erythematous swelling.[42] It is common in woman and in finger nails. Causative agent being *C.albicans,C.parapsilosis* and *C.guillermondii*. Secondary bacterial infection is a serious complication in diabetic patients.[34] (Scher,1990).It first attacks the soft tissue around the nail and then invades the nail plate.[34]

b)Onychia:

It is the involvement of the finger nail by candida species. The nail is discoloured, eroded, brittle and finally detach from the nail bed and is painful.[34]

c)Onychomycosis:

It causes total dystrophic onychomycosis. Distal nail infection presents as onycholysis [Elewski et al1995] and subungual hyperkeratosis. In contrast to dermatophytosis, candidiasis affects commonly fingernails.

DIAGNOSIS

- Direct microscopy
- Isolation in cultures
- Biochemical identification
- Phenotypic identification
- Molecular methods

DIRECT MICROSCOPY

Skin scrappings, nail clippings are examined by

- Wet mount
- KOH mount,
- Gram staining
- Calcoflour white stain
- Haematoxylin and eosin staining
- Gomori's methanamine silver stain

ISOLATION IN CULTURES

- SDA with antibiotics
- SDA with antibiotics and cycloheximide
- Both incubated at incubated at 28^0 &37^0 C.Colonies appear in two to three days.

MORPHOLOGY

Macroscopy: Smooth,creamy,pasty,glistening colony.

Microscopy: Globose, or short ovoid cells

SPECIATION OF CANDIDA

Chrom Agar

- It is used for selective isolation and speciation of yeasts
- Presumptive identification of the candida species.

This media contains chromogenic substrates. These reacts with the enzymes secreted by the yeast and produce colonies of varying colors when incubated at 37^0 C for 48-72hrs.

OTHER METHODS

- Corn meal agar and Rice starch agar-for the formation of chlamydospores.
- SDA broth
- Temperature differential studies
- Assimilation of sugar
- Fermentation of sugar
- α Glucosidase activity
- Nucleic acid hybridization studies.

Germ tube test: To differentiate C.albicans from other non albicans candida.

BIOCHEMICAL CHARACTERIZATION

Sugar assimilation tests:

It determines the ability of a yeast to utilize a specific sugar as its sole source of carbon in the presence of oxygen.

Sugar fermentation test:

It is a liquid media supplemented with different carbohydrates .The acid production and gas formation are assessed.

MOLECULAR METHODS:

- Electrophoretic pattern of DNA
- RNA profiling
- Restriction enzyme analysis
- PCR

ANTIFUNGAL SUSCEPTIBILITY TESTING

Broth dilution (CLSIM27A3)-Macrodilution and Microdilution

- Medium-RPMI1640;pH-7.0
- Inoculum-1000cells/ml
- Incubated at 35^0 C for 48hrs.

AGAR DILUTION

- Disk diffusion (CLSIM44A)-fluconazole and voriconazole.
- E-test

COLORIMETRIC METHOD

- Neosensitab
- Fungitest

FLOW CYTOMETERY

TREATMENT

Topical preparations

- 1% Gentian violet
- Nystatin
- Azoles-cream-ketoconazole,clotrimoxazole,miconazole and econazole

Oral drugs

- Ketoconazole
- Fluconazole
- Itraconazole

IMMUNOTHERAPY [45pg267]

For azole resistant strains, especially non albicans candida,antibody to SAP, mannan and mannoprotein had been tried.[walker et al2000,Mathema et al 2001,Feris 2002]

PREVENTION[34]

Reinfection with superficial candidiasis can be prevented by minimising the predisposing condition,by drying the affected areas thoroughly to avoid maceration in susceptible patients.

VACCINE [21]

Though various fungal extracts like live,attenuated and killed preparations as well as subcellular components were explored,no clinically available immunogens of proven efficacy has been obtained.

NON DERMATOPHYTE MOULDS

A few NDM, have the ability to invade keratinised tissue and produce infection that clinically resemble dermatophytoses. In immunocompromised host (DM) it causes 10-15% of onychomycosis,due to Scopulariopsis brevicaulis, Scytalidium dimidiatum, Aspergillus spp and Fusarium spp.Unlike dermatophytes, these moulds are not contagious and are common in toenails.

Scytalidium dimidiatum and Scytalidium hyalinum can invade skin and nails. It is acquired from plants or soil and occur in endemic areas.Scytalidium hyalinum is an albino variant that has not been recovered from the environment.[34] Scopulariopsis brevicaulis, a soil parasite with world-wide distribution and causes onychomycosis by invading damaged or traumatised nail.[34]

Other non dermatophyte moulds includes,Aspergillus flavus, Aspergillus fumigatus, [55], Acremonium spp and Fusarium spp which also causes onychomycosis.Aspergillus species causes 2% of onychomycosis and which causes proximal subungual paronychia without pus.[56]

Onychomycosis caused by Fusarium spp and Acremonium species are routinely not speciated (McAleer 1981, Velvez and Diaz 1985). But recently

it has been speciated as F.solani, F.oxysporum and Ac.strictum in immunosuppressed individuals [34].

Rhizopus species causes onychomycosis in immunosuppressed patients especially diabetes which appear as yellow tan demarcated area of discolouration in the nail.It needs total avulsion of the affected nail to clear the infection completely.[57]

PATHOGENESIS

Non dermatophyte moulds infect keratinised tissues of immnunocompetent host and produce keratinases (Hoeprich et al,1994). Clinically they resemble anthropophilic dermatophytes.[34]

LABORATORY DIAGNOSIS

WET KOH MOUNT

Hyaline septate hyphae of S.hyalinum resembles dermatophytes. Hence culture identification in actidione free media is essential to isolate [34]. S.dimidiatum shows phaeoid septate hyphae that can be easily differentiated from dermatophytes.

OTHER NDM

Malassezia furfur is a common commensal organism that colonise the normal skin of the head and trunk during late childhood.[33].It is uncommon in diabetic patients [7]

Tinea nigra is a chronic infection of palms and soles. The etiological agent.is Phaeoannellomyces werneckii, which is a saprophytic mould. Human

infection is by traumatic inoculation.Black piedra, is an uncommon hair infection occurring in tropical regions.

MANAGEMENT

Nondermatophyte moulds are difficult to eradicate but itraconazole is effective in nail infections caused by Aspergillus spp,Fusarium spp and Scopulariopsis brevicaulis with mycological and clinical cure of 88%.[20] Topical Amorolfine Lacquer (Downs et al 1999) with oral drugs are effective for S.hyalinum. [45pg244]

AIMS AND OBJECTIVES

- To study the prevalence of superficial mycoses in Diabetes Mellitus.
- To isolate and identify the causative organisms of superficial mycoses .
- To evaluate the minimum inhibitory concentration (MIC) and the antifungal susceptibility pattern of the isolates.

MATERIALS AND METHODS

ETHICAL CONSIDERATION

The study was conducted after obtaining approval from the institutional ethical committee of Madras Medical college, RGGGH, Chennai. Permission to conduct this study was received from the Institute of Diabetology and Institute of Dermatology .Informed consent was obtained from the patients before their enrollment in the study.

STUDY PERIOD

One year from October 2011 to September 2012.

Study setting

Institute of Microbiology, Madras Medical College, Chennai.

STUDY DESIGN

Study group

All diabetic patients attending diabetology and dermatology OPD and those admitted with fungal infections of skin,hair and nail.

Sample size-150

Inclusion criteria

All diabetic patients (type I and II) presenting with clinical features suggestive of superficial mycoses.

Exclusion criteria

- Patients on immunosuppressive drugs

- Patients on Systemic antibiotic therapy
- Patients on oral contraceptive pills
- Patients on long term steroids
- Autoimmune disorders.

HISTORY ELICITED FROM THE PATIENT

All the relevant details from the patient were elicited using preformed proforma.(Annexure)

COLLECTION OF SAMPLE [20]

Samples were collected from all patients who gave history of skin, scalp or nail lesions clinically diagnosed as superficial fungal infection.

- Skin scraping
- Nail clipping
- Plucked hair.
- **Skin:** The lesion was thoroughly cleaned with 70% alcohol and allowed to dry. Skin lesion was scrapped from periphery (active margin) using flame sterilized blunt scalpel without injuring the skin surface.
- Strongly macerated skin between toes was removed by forceps.
- Samples from vesicles was collected by gently deroofing with a needle.

- **Hair**: The same procedure as above was followed as for skin scrapings, in addition a few affected hairs were also epilated and collected with a pair of flame sterilized tweezers. Care was taken to collect the basal portion of the hair

- **Nail**: The affected nail was meticulously swabbed with 70% alcohol .Discolored, dystrophic or brittle nails were clipped as far as back as possible from the free edge including the full thickness and scrapings were collected from the white spots [66,p884-5]

TRANSPORT OF SAMPLES:[44]

- The samples were collected in a sterile piece of black card board (six inches square,folded to form an envelope) which was clipped together,labelled and transported.

- Interdigital space lesions were cleaned with sterile saline and collected in sterile swabs and inoculated immediately in culture media.

PROCESSING OF SAMPLE:[20,43,44,53]

1)Direct Microscopic examination:

Direct microscopic examination was done for all the samples.

Procedure

- A large drop of 10% KOH (skin/hair) or 20% KOH (nail) solution (Annexure) was placed with a pastuer pipette on a clean grease free glass slide .

- A part of the sample was transferred into the KOH on the slide.

- A clean cover slip was placed gently over the mixture without the formation of any air bubbles.
- The slide was kept at room temperature for 20-30mins (skin/hair samples) or overnight.(nail sample).
- The specimen was observed under low and high power objectives.

Observation

The type of hyphae, septation, branching,thickness, arthroconidia and other features were studied.

Yeasts were identified by observing the presence of oval to elongated yeast cell with or without pseudohyphae.

CULTURE [20,43,44,53]

All samples were cultured irrespective of the direct microscopic observation.

Media

The scrapings were inoculated in duplicate sets into slopes containing,

a) SDA with chloramphenicol/gentamicin and

b) SDA with chloramphenicol/gentamicin and cycloheximide. (Annexure).

One set of SDA was incubated at 37^0 C and second set at 25^0 C.

c) One set of DTM containing chloramphenicol/gentamicin and cycloheximide (Annexure) was also inoculated and incubated at 25^0 C.

IDENTIFICATION OF FUNGAL ISOLATES [41,66]

Any visible growth on either of the slants was examined for,

1) Colony morphology (yeast/Mould]) [43]

   a. Topography

   b. Texture

   c. Surface pigmentation

   d. Pigmentation on reverse

   e. Presence of diffusible pigment

   f. Rate of growth.

All the SDA tubes were read every day for a week following incubation and twice weekly thereafter for 4-6weeks.[20] DTM tubes are read for growth and colour change in the medium daily for 7-10 days.[20,46] Tubes showing no growth after the specified period were discarded.

MICROSCOPY [20,44]

a.**Gram staining**(Annexure) :Creamy white colonies with features suggesting of yeast were Gram stained .

Procedure

- Tiny part of a colony was picked and a smear was prepared.
- The smear was allowed to dry and heat fixed.
- The smear flooded with Methyl violet for one minute and excess stain poured off.
- The slide was rinsed in flowing tap water.
- Gram's Iodine was added and rinsed in flowing tap water after one minute.
- Acetone was added and rinsed off with tap water after 2-3secs.
- Finally the smear was counterstained with dilute carbol fuschin for few secs.
- The slide was washed in tap water and air dried.
- The smear was observed under oil immersion objective.

OBSERVATION:

Gram reaction, size, shape and arrangement of cells were observed.

INTERPRETATION

Gram positive oval to elongated cell-Yeast cell.Presence or absence of pseudohyphae are noted.

LACTOPHENOL COTTON BLUE (LPCB) [45] (ANNEXURE)

LPCB mount was prepared from colonies resembling mould and examined for hyphal morphology and its pigmentation,conidia morphology (microconidia and macroconidia) and its arrangements.Lactic acid aids in preserving the fungal structure;phenol acts as a disinfectant and cotton blue imparts color to the structures.

Procedure [20,44]

- A drop of LPCB was placed on a clean dry glass slide.
- Using a straight mycological loop a tiny portion of the colony to be examined was removed from the agar and placed on the stain.
- With two dissecting needles the mycelial mass was gently well teased apart.
- A cover slip was placed and observed under low and high power objectives.

Interpretation

Morphology of the isolates was observed and the causative fungus identified [20,43,46]

SLIDE CULTURE [20,43]

The slide culture technique was done to study the typical morphology of the isolate, like arrangement of conidia, without disturbing the relationship between reproductive structures and mycelium or the sporulation characteristics of the organism.

PROCEDURE

- A slide was placed on a bent glass rod in a petridish with cover slip, filter paper and the dish was wrapped and sterilized by autoclaving at 121^0C for 15mins.
- With a sterile scalpel an agar block 1sqcm and 2-3 mm deep was cut out of a plate of SDA.This block was transferred onto the center of the

sterilised glass slide.With a heavy nichrome wire bend loop,the four corners of the agar block were inoculated with the fungal isolate under study and the block was covered with the sterilized cover slip.

- 1-1.5ml of sterile distilled water was pipetted on to the filter paper placed inside the petri dish so as to prevent drying of the agar block.

- The plate was incubated at room temperature and examined periodically for growth.Once sporulation had well developed,the cover slip was carefully separated using a sterile forceps from the agar block ,flamed quickly to fix the fungus with the spores.

- Heat fixed cover slip was placed on a drop of LPCB on a second glass slide.

- The agar block was gently flipped off the original slide, few drops of LPCB was added and new cover slip was laid over the preparation.Both the slides were then examined under microscope.When examination is likely to be delayed,the edges of the LPCB mount were sealed with nail polish to prevent drying.

GERM TUBE TEST [20,43]

Principle

The in vitro production of germ tubes by Candida albicans, when incubated in serum for two hours is noted.

Procedure

- It is performed with freshly sub cultured pure growth.

- The organism is inoculated onto 0.5ml of serum .
- Incubated at 37^0C for two hours.
- After incubation, the suspension was placed on a clean, dry slide and covered with coverslip and observed under low and high power objectives.

Interpretation

- Germ tube test was considered positive (within two hours)-if 30% of cells shows long tube like projection from a mother cell which are non septate with parallel sides and no constriction at the point of connection were presumptively identified as Candida albicans/Candida dubliniensis.
- Germ tube negative-Non albicans candida.

CHROM AGAR [20] (ANNEXURE)

This differential culture media was inoculated to speciate the candida isolates.Isolates from primary medium (SDA) were subcultured in SDA and single yeast colony was streaked onto the Chromagar plates.Incubated at 37^0 C for 48-72hrs.

Interpretation:

C.albicans	Light green
C.dubliensis	Dark green
C.glabrata	Pink to purple
C.krusei	Pink

C.parapsilosis Cream to pale pink

C.tropicalis Blue with pink halo.

BIOCHEMICAL REACTION

UREA HYDROLYSIS [43,44]

Modified Christensen's medium (Annexure) for urea hydrolysis was used to distinguish T.mentagrophytes from T.rubrum.

Procedure

The slopes were inoculated with a small amount of the fungal culture and was incubated at room temperature along with a control tube . The results were noted for 2-5days

Interpretation

- Urease positive (deep pink) within 4days -T.mentagrophytes
- Urease negative - T.rubrum.

SUGAR FERMENTATION TEST [20,43] (ANNEXURE)

- A loopful of 24-48hrs culture from a sugar free media was suspended in sterile distilled water.
- 0.1ml of this suspension was added to 2% sugar fermentation media with Bromothymol blue indicator.
- Dextrose,lactose,sucrose,maltose,galactose and trehalose were tested.
- Inoculated sugars were incubated at 25^0C for 48-72hrs.

- ❖ The ability to ferment a sugar was noted by the production of acid and the presence of gas in Durham's tube.

INTERPRETATION [43]

Species	Dextrose	Lactose	Sucrose	Maltose	Galact	Trehal
C.albicans	F	NF	NF	F	F	F
C.tropicalis	F	NF	F	NF	NF	NF
C.parapsilosis	F	NF	NF	NF	NF	NF
C.glabrata	F	NF	NF	NF	NF	NF
C.krusei	NF	NF	NF	NF	NF	NF
C.guillermondii	NF	NF	NF	NF	NF	NF
C.kefyr	NF	F	F	NF	F	NF

F-Fermented NF-Not fermented

IN VITRO HAIR PERFORATION TEST [43,46]

Hair perforation test was done to differentiate between T.mentagrophytes and T.rubrum.Growth of the fungus in artificial culture media along with the hair was observed.

Procedure

- ❖ 1cm human hair from a child <5years was placed in a petridish and autoclaved at 121^0C for 10mins.
- ❖ To it 25ml of sterile distilled water , 2-3drops of filter sterilized 10% yeast extract (Annexure)were added.
- ❖ The plate was inoculated with a small fragment of the test fungi.
- ❖ The plate was incubated at room temperature (25^0C) for 4weeks.

❖ Periodically each hair strands were removed, mounted on a slide containing LPCB and examined under low/high power objectives.

Interpretation

- Positive (wedge shaped perforation)-T.mentagrophytes and all Microsporum species
- Negative-T.rubrum.

ANTIFUNGAL SUSCEPTIBILITY TESTING [46]

Microbroth dilution method: Evaluation of MIC

Antifungal agents

Source: Antifungal standards or reference powder with its assay potency in µg/ml or IU/mg.

Weighing antifungal powders: Can be derived by using either of the formulae.

$$\text{Weight(mg) of drug} = \frac{\text{Vol(ml)XConc(µg/ml)}}{\text{Assay potency (µg/mg)}}$$

$$\text{Volume of Diluent(ml)} = \frac{\text{Weight(mg) X Assay potency (µg/mg)}}{\text{Conc: (µg/ml)}}$$

Preparation of stock solution

❖ Antifungal stock solutions were prepared at a concentration of 1280µg/ml for water soluble drug and 1600µg/ml for water insoluble drug.

- Water soluble drug like Fluconazole was dissolved in distilled water and two dilution was prepared as given in Chart 1.

- Water insoluble drugs, Amphotericin B, Itraconazole, Ketoconazole, Voriconazole and terbinafine were dissolved DMSO and diluted with RPMI 1640.Dilution were prepared following Chart-2.

Number of concentrations tested

- Amphotericin B 0.0313 to 16µg/ml
- Ketoconazole 0.0313 to 16µg/ml
- Itraconazole 0.0313 to 16µg/ml
- Voriconazole 0.0313 to 16µg/ml
- Fluconazole 0.125 to 64µg/ml
- Terbinafine 0.0313 to 16µg/ml

Medium: Rosewell Park memorial institute (RPMI) 1640(Annexure)with glutamine without bicarbonate.

Buffer: MOPS (3-N-Morpholino prophanesulfonic acid)

CHART-1 :SCHEME FOR PREPARING DILUTIONS OF WATER SOLUBLE DRUGS

Drug(µg/ml)	Stock 5120	2	4	8	16	32	64	128	256	512	Remark
TUBE	TUBE 1	2	3	4	5	6	7	8	9	10	
SOURCE DRUG(ml)	From stock 1.0	T-1 1.0	T-1 1.0	T-3 1.0	T-3 0.5	T-3 0.5	T-6 1.0	T-6 0.5	T-6 0.5	T-9 1.0	Step 1 Row 1
RPMI 1640	7.0	1.0	3.0	1.0	1.5	3.5	1.0	1.5	3.5	1.0	
INTERMEDITE DRUG CONC.(µg/ml)	640	320	160	80	40	20	10	5	2.5	1.25	
Add drug from Row1(ml)+RPMI(ml)	1+4	1+4	1+4	1+4	1+4	1+4	1+4	1+4	1+4	1+4	Step 2 Row 5x(1:4)
Final concentration 1:5(µg/ml)	128	64	32	16	8	4	2	1	0.5	0.25	2X
From Row 2 add drug to plate(ml)	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	Step 3 1:1
Inoculum (ml) to plate	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	Step 4
Final drug conc: In each well 1:100 µg/ml)	64	32	16	8	4	2	1	0.5	0.25	0.125	

T= Tube

CHART 2 :SCHEME FOR PREPARING DILUTIONS OF WATER INSOLUBLE DRUGS

Drug(µg/ml)	Stock	2	4	8	16	32	64	128	256	512	Remark
TUBE	TUBE1	2	3	4	5	6	7	8	9	10	
SOURCE DRUG(ml)	From stock 1.0	T-1 0.5	T-1 0.5	T-1 0.5	T-4 0.5	T-4 0.5	T-4 0.5	T-7 0.5	T-7 0.5	T-7 0.5	STEP 1 ROW 1
Solvent DMSO(ml)	-	0.5	1.5	3.5	0.5	1.5	3.5	0.5	1.5	3.5	
INTERMEDITE DRUG CONC.	1600	800	400	200	100	50	25	12.5	6.25	3.13	
Adddrug from T1Row1(ml)+RPMI(ml)	0.1+4.9	0.1+4.9	0.1+4.9	0.1+4.9	0.1+4.9	0.1+4.9	0.1+4.9	0.1+4.9	0.1+4.9	0.1+4.9	STEP 2 ROW2 (1:50)
Final concentration 1:50(µg/ml)	32	16	8	4	2	1	0.5	0.25	0.125	0.0625	2X
From Row 2 add drug to plate(ml)	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	STEP 3 1:1
Inoculum (ml)	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	STEP 4 T.Vol0.2ml
Final drug conc: In each well 1:100 (µg/ml)	16	8	4	2	1	0.5	0.25	0.125	0.0625	0.0313	

T-Tube

YEAST (CANDIDA):CLSI M27-A 2nd Ed [46]

Reference strain-Candida albicans ATCC ®90028

Test strains: Isolated Candida species

Preparation of inoculum

- All isolates were subcultured onto SDA.
- After 48hrs,five identical colonies of 1mm (dia) were picked and suspended in 5ml of sterile normal saline.
- The suspension was vortexed for 15secs and the cell suspension was adjusted to 0.5McFarland standards (1-5x10^6cells/ml).
- A working suspension of 1:5 (1-5x10^3CFU/ml) was prepared using RPMI as diluent.

Drugs tested

- Amphotericin B
- Fluconazole
- Voriconazole
- Itraconazole

Procedure of microbroth dilution

- 100µl of each drug added through wells 1 to 10 according to the concentration prepared.
- 100µl of the inoculum added to all wells making a final concentration of inoculum to 0.5-2.5x10^3 CFU/ml.

- The microtiter plates were kept at 35^0 C for 48hrs.

Interpretation: MIC for azoles was determined by noting 80% inhibition of growth while for Amphotericin B, 100% growth inhibition.

DRUG	MIC range µl/ml	Sensitive	Resistant
Amphotericin B	0.0313-16	≤1µg/ml	>1µg/ml
Fluconazole	0.125-64	≤8µg/ml	>8µg/ml
Itraconazole	0.0313-16	≤0.25µg/ml	>0.25µg/ml
Voriconazole	0.0313-16	≤1µg/ml	>1µg/ml

DERMATOPHYTES AND NON DERMATOPHYTES MOULDS

***METHOD*:** CLSI M-38A 2nd edition was followed.[46,58,59]

Reference strain

- C.parapsilosis ATCC® 22019-Dermatophytes[90-92]

KORTING, H.C.;et al(1995) stated that no standard reference method for determination of MIC to dermatophyte is available, hence broth microdilution method (CLSI M38A)which gives good correlation with clinical outcome can be followed.

- A.flavus ATCC® 204304-Non dermatophytic moulds

Test strains: All isolated dermatophytes and non dermatophyte moulds

Preparation of inoculum

- All strains were freshly subcultured onto SDA

- After colonies had well grown in the tube, they were filled with 10 ml of distilled water.

- The inoculum was prepared by scraping the surface of the colonies with the tip of a sterile loop.

- The inoculum thus obtained was transferred to another sterile tubes and left for 15 to 20 minutes at room temperature to sediment the heavy particles.

- The optical density of the suspensions containing conidia and hyphal fragments was read at 530 nm, adjusted to transmittance of 65 to 70% (2 to 4 X 10^6 cells/mL) and further diluted with RPMI 1640 medium to obtain the final inoculum size of 0.4 to 5 X 10^4 cells/mL.

Drugs tested

- Terbinafine
- Ketoconazole
- Itraconazole
- Voriconazole
- Amphotericin B

Procedure of microbroth dilution

- 100µl of each drug added through wells 1 to 10 according to the concentration prepared.

- 100µl of the inoculum added to all wells making a final concentration of inoculum to 0.2-2.5x10^4 CFU/ml.

- Incubation temperature & time for dermatophytes-24^0C/4-5days

❖ Incubation temperature & time for Non dermatophytes-35^0 C/24-48hrs.

Interpretation

MIC for azoles was determined by noting 80% inhibition of growth while for terbinafine, 100% growth inhibition.

DRUGS	MIC range µl/ml	Sensitive	Resistent
Terbinafine	0.0313-16	≤0.25	>0.25
Itraconazole	0.0313-16	≤0.5*	>0.5*
Voriconazole	0.0313-16	≤0.250	>0.250
Amphotericin B	0.0313 -16	≤2	>2
Ketoconazole	0.0313 -16	≤2	>2

* For NDM MIC=≤1.0 (S);>1.0(R)

RESULTS

TABLE-1: AGE WISE DISTRIBUTION OF STUDY POPULATION (n=150)

Age group (years)	No.of cases (n)	Percentage (%)
21-30	2	1.3
31-40	24	16
41-50	52	34.6
51-60	38	25.3
61-70	18	12
71-80	14	9.3
81-90	2	1.3

The study population consisted of patients in the age of 26-85 years. There was preponderance of cases in the 41-50years age group population. (p=0.8315,Fischer's exact test)

Fig-1: Age Distribution

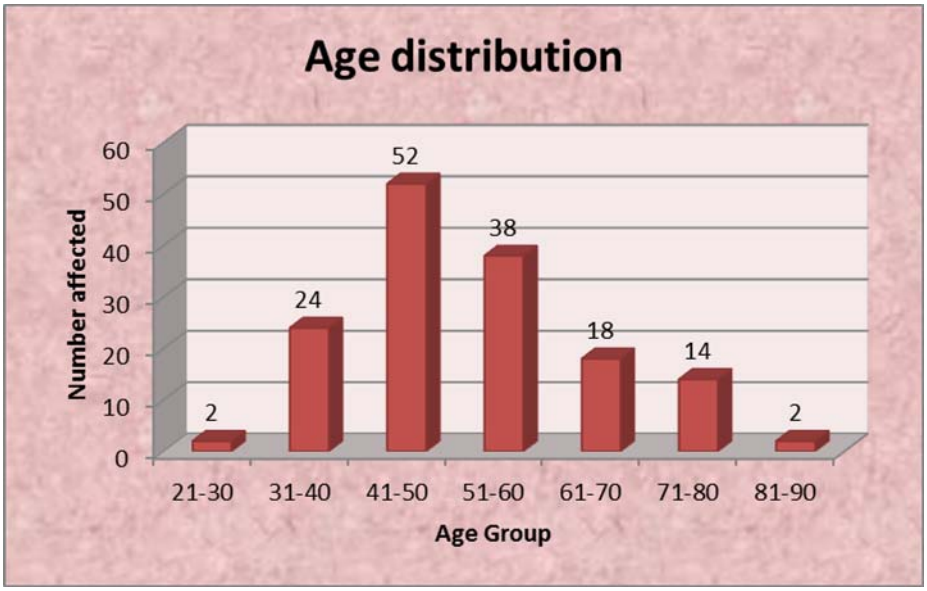


TABLE:2 GENDER WISE DISTRIBUTION OF STIDY POPULATION (n=150)

	Male	Percentage	Female	Percentage	Total	Ratio (M:F)
No.of case(n)	78	52%	72	48%	150	1.06:1

The study population consisted of 78 (52%) males and 72(48%) females and the Male:female ratio was 1.06:1.(p=0.1410.Fischer's exact test)

Fig-2: Male Vs Female

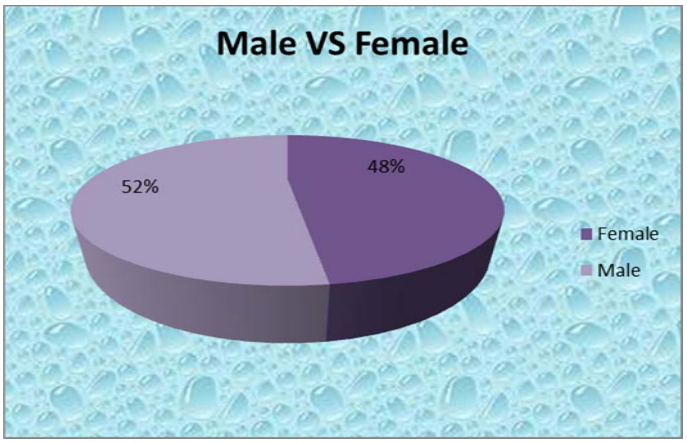


TABLE-3: DISTRIBUTION OF DIABETES MELLITUS IN STUDY POPULATION(N=150)

	Type-I	Percentage	Type-II	Percentage	Total
No.of case(n)	18	12%	132	88%	150

Type II diabetes were more affected than the type I.(p=0.6190,Fischer's exact test)

Fig-3: Type of Diabetes

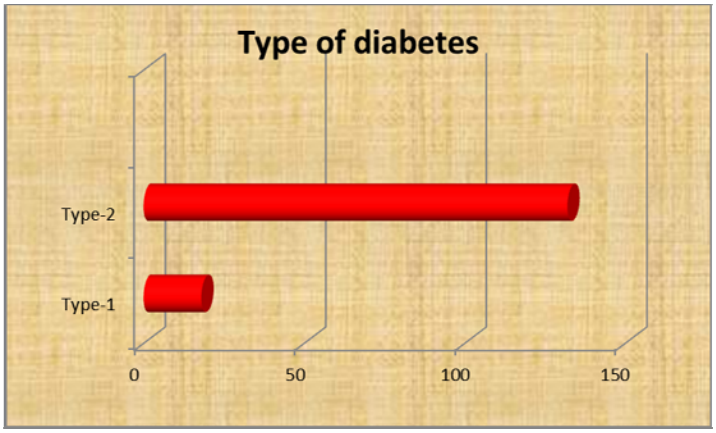


TABLE-4: SAMPLES COLLECTED FROM STUDY POPULATION (n=150)

S.No	Specimen	No.of sample collected (n)	Percentage (%)
1.	SKIN	63	42
2.	NAIL	87	58
	TOTAL	150	100

The total samples collected from skin scrappings were 63(42%)and from the nail clippings 87 (58%).

Fig-4: Skin Vs Nail

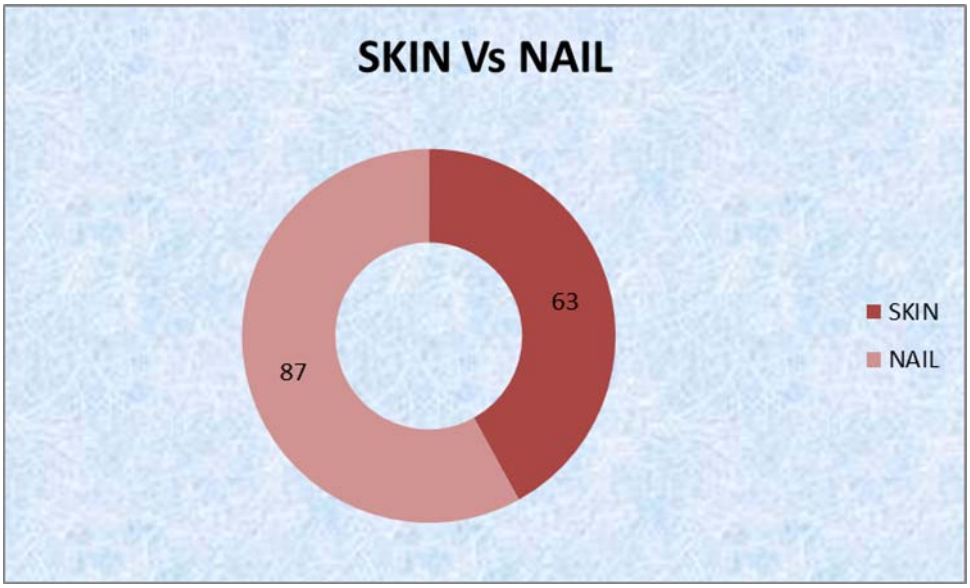


TABLE-5: DISTRIBUTION OF DERMATOMYCOSIS IN THE STUDY POPULATION (n=63)

S.No	Clinical classification	No.of.case(n)	Percentage%
1.	Tinea capitis	0	0
2.	Tinea faciei	1	1.5
3.	Tinea barbae	1	1.5
4.	Tinea corporis	40	63.4
5.	Tinea manuum	2	3.1
6.	Tinea cruris	14	22.2
7.	Tinea pedis	2	3.1
8.	Web space	3	4.7
	TOTAL	**63**	

The predominant dermatomycotic lesion was tinea corporis which accounted for 40 (63.4%) cases followed by tinea cruris 14(22.2%),tinea pedis 2(3.1%) and web space infection 3(4.7%).

Fig-5: Skin Lesion

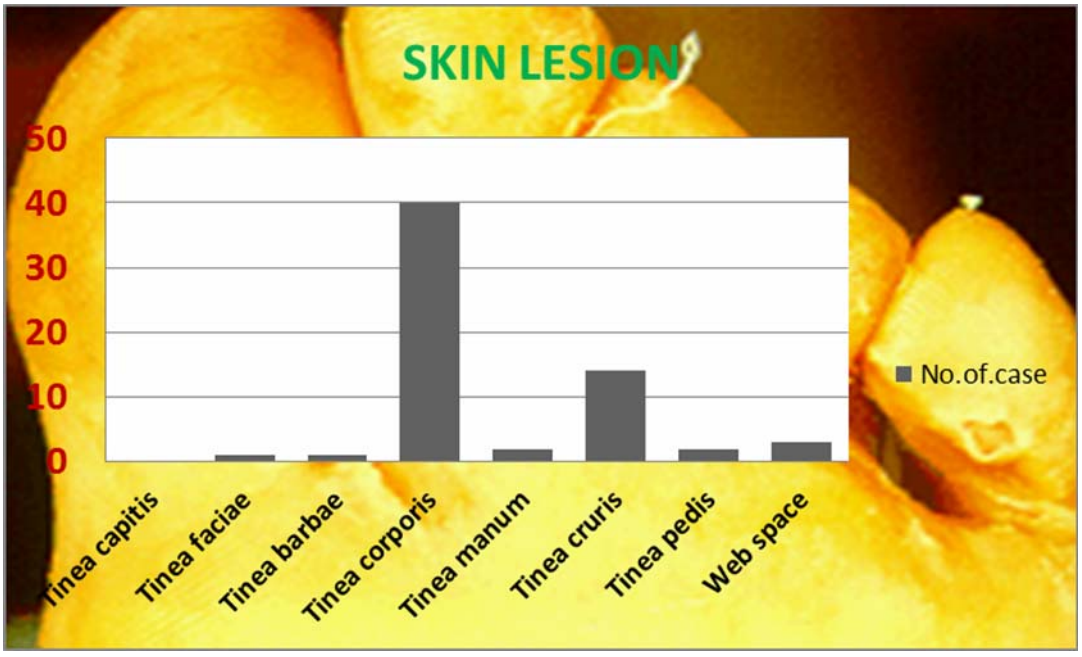


TABLE-6: DISTRIBUTION OF ONYCHOMYCOSIS IN THE STUDY POPULATION(n=87)

S.No	Clinical classification	No.of cases(n)	Percentage(%)
1.	DLSO	58	66.6
2.	PSO	3	3.4
3.	WSO	21	24.1
4.	TDO	5	5.7
	TOTAL	**87**	

The predominant onychomycotic lesion was DLSO which accounted for 58(66.6%) cases followed by WSO in 21 (24.1%) cases.

Fig-6: Nail Lesion

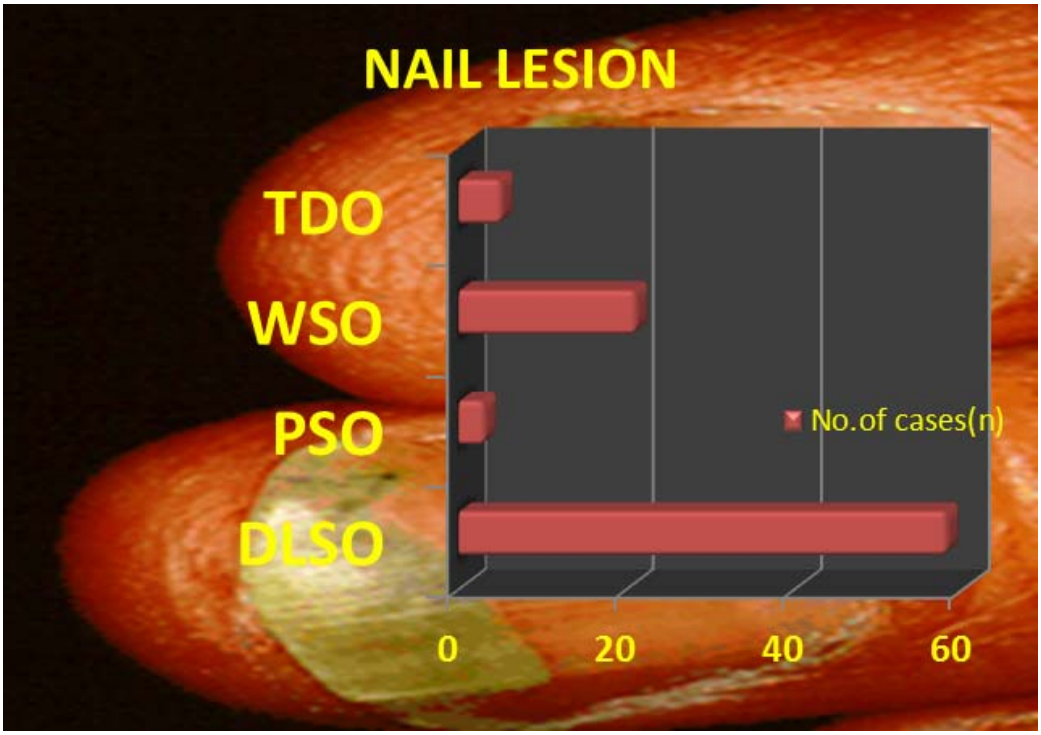


TABLE-7: EVALUATION OF KOH MOUNT(n=150)

S.No	SAMPLE	KOH Positive(%)	KOH Negative(%)	TOTAL
1.	SKIN	35	28	63
2.	NAIL	30	57	87
	TOTAL	**65 (43)**	**85 (56)**	**150**

The direct microscopic examination (KOH) was positive in 65 (43%) samples and was negative in 85 (56%) samples.

Fig-7: Samples Vs KOH

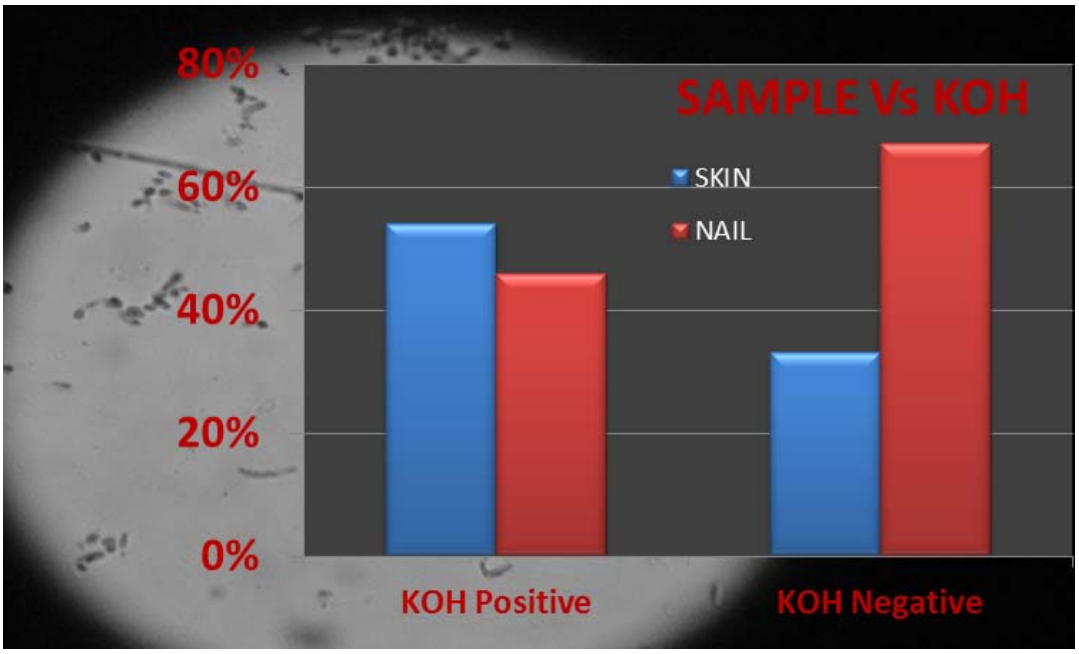


TABLE-8: EVALUATION OF CULTURE(n=150)

S.No	SAMPLE	Culture Positive(%)	Culture Negative(%)	TOTAL
1.	SKIN	39	24	63
2.	NAIL	34	53	87
	TOTAL	**73 (48)**	**77 (51)**	**150**

The total number of samples which showed growth in cultures were 73(48%) and 77 (51%) samples showed no growth in cultures.

Fig-8: Sample Vs Culture

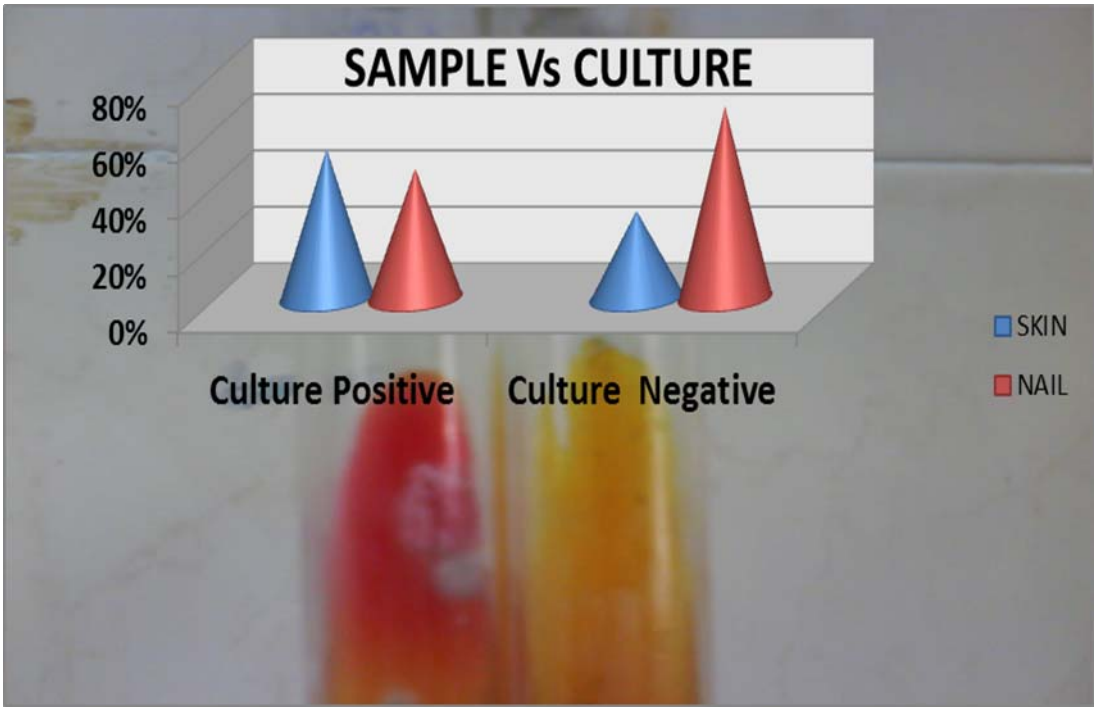


TABLE-9: CORRELATION BETWEEN DIRECT MICROSCOPY (KOH) AND CULTURE (n=150)

S. No	Lesion	Koh+ve culrure +ve	Koh+ve culture -ve	Koh-ve culture +ve	Koh-ve culture -ve	Total
1.	Tinea faciei	-	-	1	-	1
2.	Tinea barbae	-	-	1	-	1
3.	Tinea corporis	13	2	7	18	40
4.	Tinea manuum	1	-	1	-	2
5.	Tinea cruris	6	2	4	2	14
6.	Tinea pedis	1	-	-	1	2
7.	Web space	-	-	2	1	3
8.	Tinea Unguium	14	24	20	29	87
TOTAL		**35 (23.3%)**	**28 (18.6%)**	**36 (24%)**	**51 (34%)**	**150**

A total of 35(23.3%) samples were positive on direct examination and culture and 51(34%) samples were negative by both the tecniques.Samples positive by direct examination and negative on culture was 28(18.6%). Further 36 (24%) samples isolated on culture were negative on direct examination. (p=0.4175;Fischer exact test)

Fig-9:KOH Vs Culture

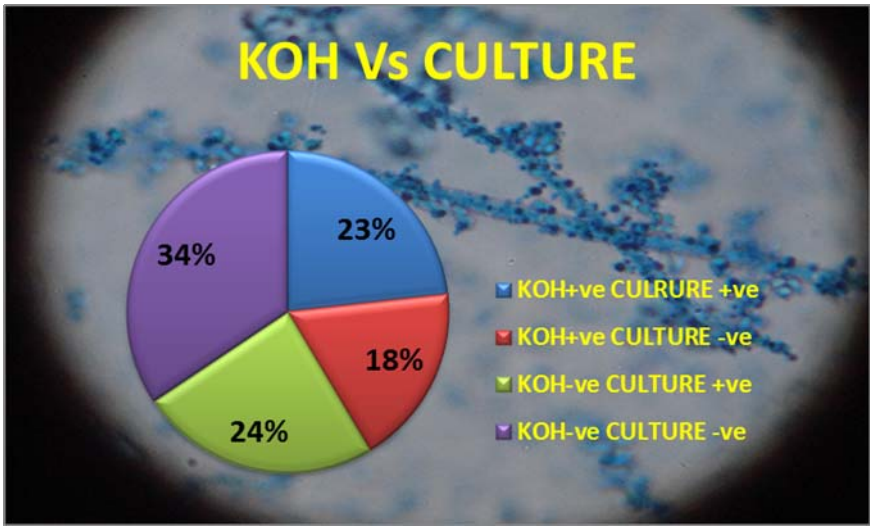


TABLE-10: ISOLATES OF SUPERFICIAL MYCOSES IN THE STUDY POPULATION (n=73)

S.No	ISOLATES	SKIN	NAIL	TOTAL	Percentage
1.	DERMATOPHYTE	33(84%)	14(41%)	47	64.3%
2.	CANDIDA	6(15%)	11(32%)	17	23.2%
3.	NONDERMATOPHYTE	0	9(26%)	9	12.3%
TOTAL		**39**	**34**	**73**	

The predominant pathogen isolated from skin and nail was dermatophytes followed by candida species.Non dermatophyte moulds were isolated from nail lesions.

Fig-10a: Skin Lesion Culture

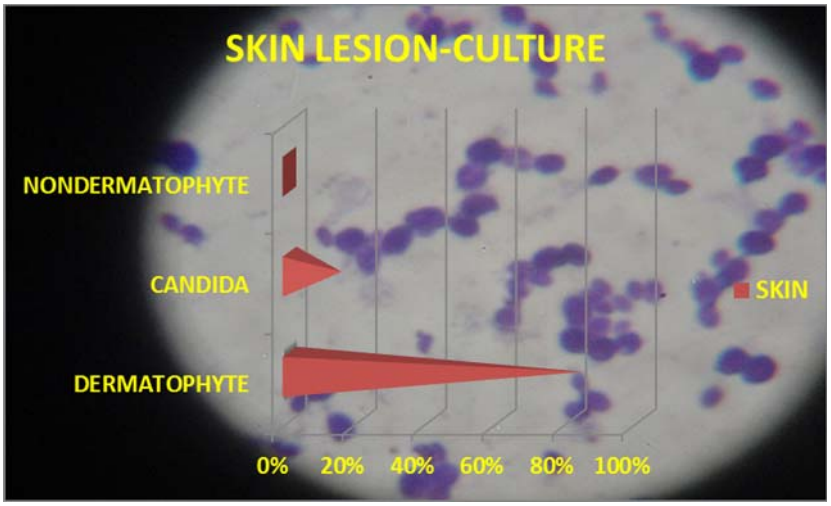


Fig-10b: Nail Lesion Culture

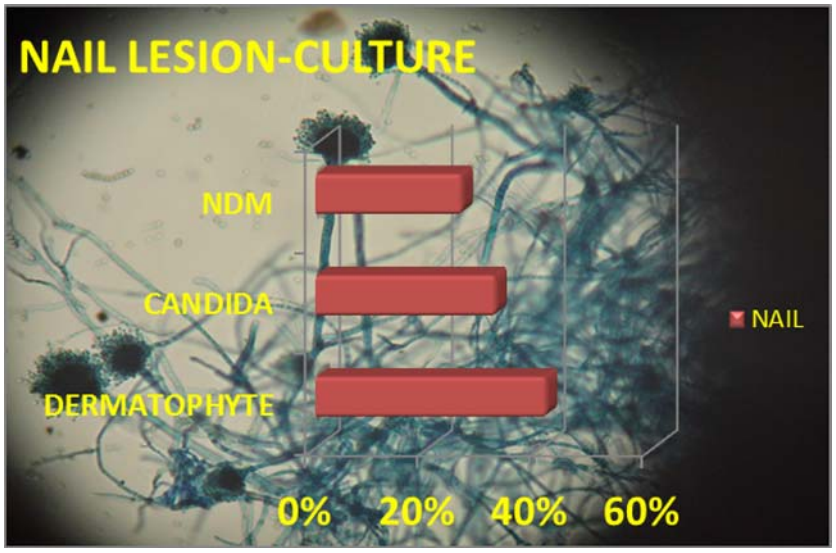


TABLE-11: CLINICOMYCOLOGICAL PATTERN OF SUPERFICIAL MYCOSES (n=73)

S. No	Clinical lesion	Dermatophytes				Candida			Nondermatophytes			
		T.m	T.r	T.t	T.v	C.p	C.t	C.g	Rhi	Fus	A.f	A.n
1.	Tinea faciei	_	1	_	_	_	_	_	_	_	_	_
2	Tinea barbae	1	_	_	_	_	_	_	_	_	_	_
3	Tinea corporis	15	4	1	1	_	_	_	_	_	_	_
4	Tinea manuum	1	_	_	_	_	1	_	_	_	_	_
5	Tinea cruris	3	3	_	1	1	2	_	_	_	_	_
6	Tinea pedis	1	1	_	_	_	_	_	_	_	_	_
7	Web space	_	_	_	_	_	1	1	_	_	_	_
8	Tinea unguium	12	2	_	_	1	7	3	4	2	2	1
TOTAL		**33**	**11**	**1**	**2**	**2**	**11**	**4**	**4**	**2**	**2**	**1**
Percentage(%)		45.2	15	1.3	2.7	2.7	15	5.4	5.4	2.7	2.7	1.3

The predominant isolate among the dermatophyte was Trichophyton mentagrophytes which accounted for 33 (45%) followed by Trichophyton rubrum in 11(15%) cases.The predominant isolates among candida was candida tropicalis which accounted for 11(15%) and in nondermatophyte moulds it was Rhizopus 4(5.4%) followed by Fusarium and A.fumigatus 2(2.7%) cases each.

Fig-11 (a): Dermatophytes

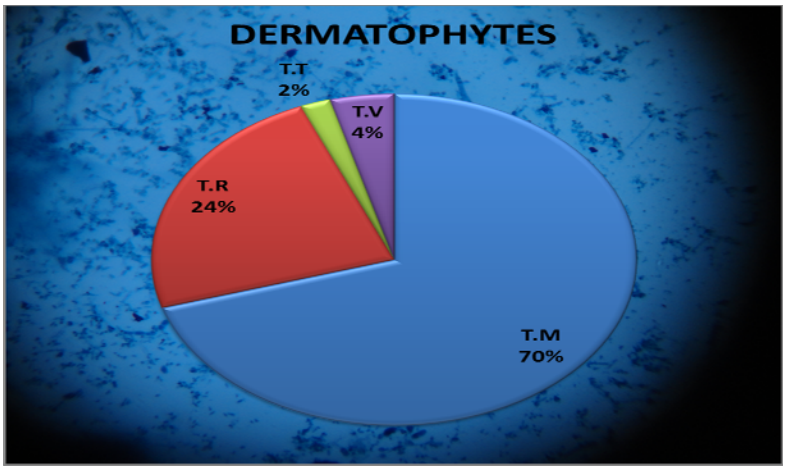


Fig-11 (b): Candida

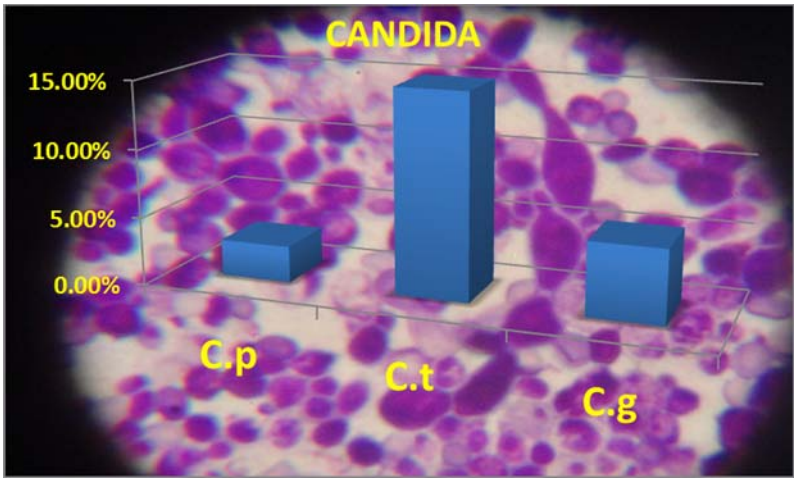


Fig-11 (c): Non Dermatophyte Mould

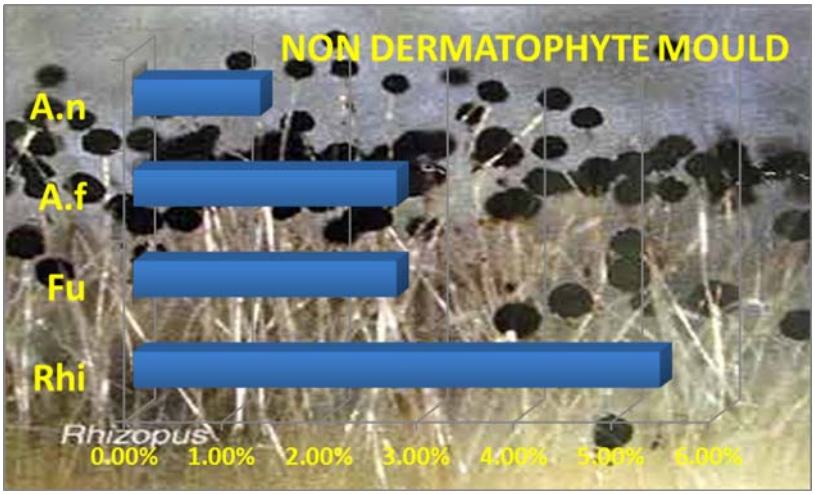


TABLE-12: ANTIFUNGAL SUSCEPTIBILITY PATTERN (MIC) OF TERBINAFINE TO THE ISOLATED DERMATOPHYTES (n=47)

S.No	STRAIN	No.of.Isolates	Sensitive MIC≤0.250	Resistence MIC>0.250	MIC µg/ml
1.	C.parapsilosis ATCC® 22019	1	1(100%)	_	0.125
2	T.mentagrophytes	33	33(100%)	_	0.0625-0.250
3	T.rubrum	11	9(81%)	2*(18%)	0.0313-0.125
4	T.tonsurans	1	1(100%)	_	0.0625
5.	T.verrucosum	2	2(100%)	_	0.0625-0.125

All isolates were sensitive to terbinafine except for two strains of T.rubrum

***MIC=0.5µg/ml and 16µg/ml**

Fig-12

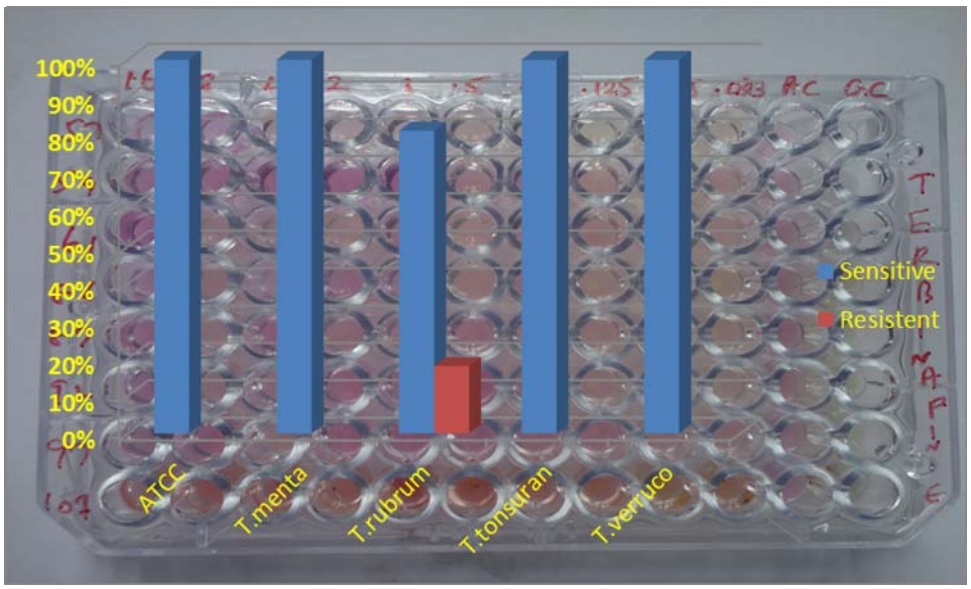


TABLE-13: ANTIFUNGAL SUSCEPTIBILITY PATTERN (MIC) OF KETOCONAZOLE TO THE ISOLATED DERMATOPHYTES (n=47)

S.No	STRAIN	No.of.Isolates	Sensitive MIC≤2	Resistence MIC>2	Mean MICµg/ml
1.	C.parapsilosis ATCC ®22019	1	1(100%)	_	0.250
2	T.mentagrophytes	33	33 (100%)	_	0.125-1
3	T.rubrum	11	10(96%)	1*(4%)	0.125-1
4	T.tonsurans	1	1(100%)	_	0.5
5.	T.verrucosum	2	2(100%)	_	0.250-1

All isolates were sensitive to ketoconazole except for one strain of T.rubrum.*MIC=4µg/ml

Fig-13

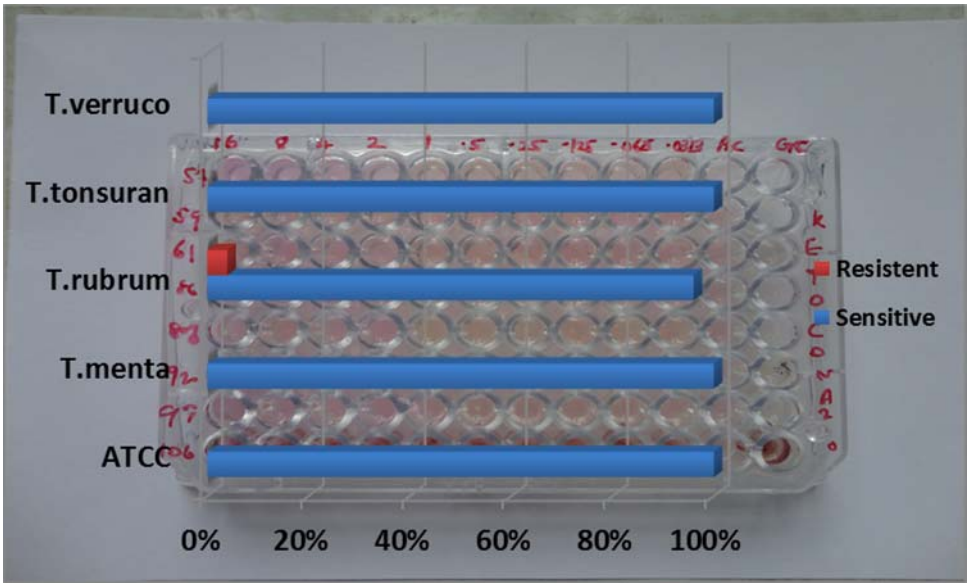


TABLE-14: ANTIFUNGAL SUSCEPTIBILITY PATTERN (MIC) OF ITRACONAZOLE TO THE ISOLATED DERMATOPHYTES (n=47) AND NONDERMATOPHYTE MOULDS (n=9)

S.No	STRAIN	No.of. Isolates	Sensitive MIC≤0.250*	Resistence MIC>0.250	Mean MICµg/ml
1.	C.parapsilosis ATCC ®22019	1	1(100%)	_	0.125
2	T.mentagrophytes	33	33 (100%)	_	0.0625-0.250
3	T.rubrum	11	10(96%)	1**(4%)	0.0313-0.125
4	T.tonsurans	1	1(100%)	_	0.125
5.	T.verrucossum	2	2(100%)	_	0.125-0.250
6	A.flavus ATCC ®204304	1	1(100%)	_	0.250
7	A.fumigatus	2	2(100%	_	0.25-0.5
8	A.nidulans	1	1(100%)	_	0.5
9	Rhizopus spp	4	4(100%	_	0.25-1
10	Fusarium spp	2	2(100%)	_	0.25-1

*For dermatophytes MIC=<0.250 µg/ml is sensitive and for nondermatophyte moulds MIC=-<1µg/ml

All isolates were sensitive to itraconazole except for one strain of T.rubrum

**MIC=16.0µg/ml.

Fig-14:

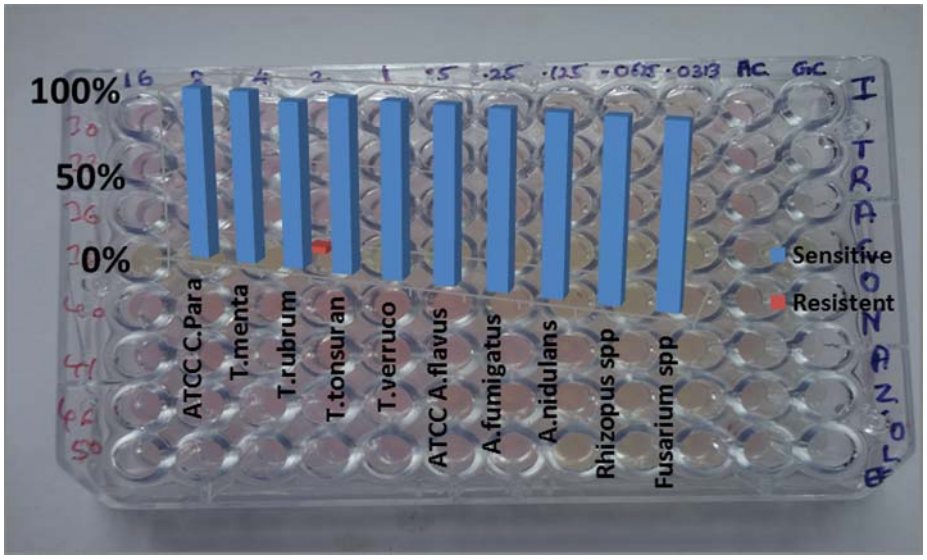


TABLE-15: ANTIFUNGAL SUSCEPTIBILITY PATTERN (MIC) OF VORICONAZOLE TO THE ISOLATED NONDERMATOPHYTE MOULDS (n=9)

S.No	STRAIN	No.of.Isolates	Sensitive MIC≤0.250	Resistence MIC>0.250	Mean MICµg/ml
1.	A.flavus ATCC ®204304	1	1(100%)	_	0.125
2	A.fumigatus	2	2(100%	_	0.0625-0.125
3	A.nidulans	1	1(100%)	_	0.250
4	Rhizopus spp	4	4(100%	_	0.125-0.250
5.	Fusarium spp	2	2(100%)	_	0.0625-0.25

All isolates were sensitive to voriconazole.

Fig-15

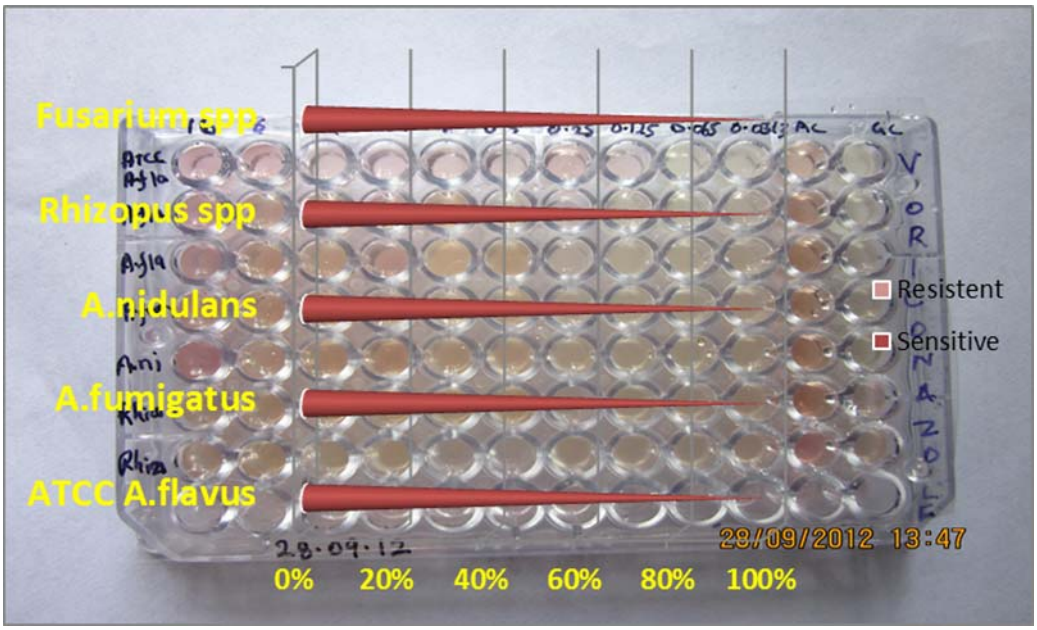


TABLE-16: ANTIFUNGAL SUSCEPTIBILITY PATTERN (MIC) OF AMPHOTERICIN B TO THE ISOLATED NONDERMATOPHYTE MOULDS (n=9)

S. No	STRAIN	No.of.Isolates	Sensitive MIC≤2	Resistence MIC>2	Mean MICµg/ml
1.	A.flavus ATCC ®204304	1	1(100%)	_	0.250
2	A.fumigatus	2	2(100%	_	0.5-1
3	A.nidulans	1	1(100%)	_	1.0
4	Rhizopus spp	4	4(100%	_	0.5-2
5.	Fusarium spp	2	2(100%)	_	0.5-1

All isolates were sensitive to amphotericin B

Fig-16

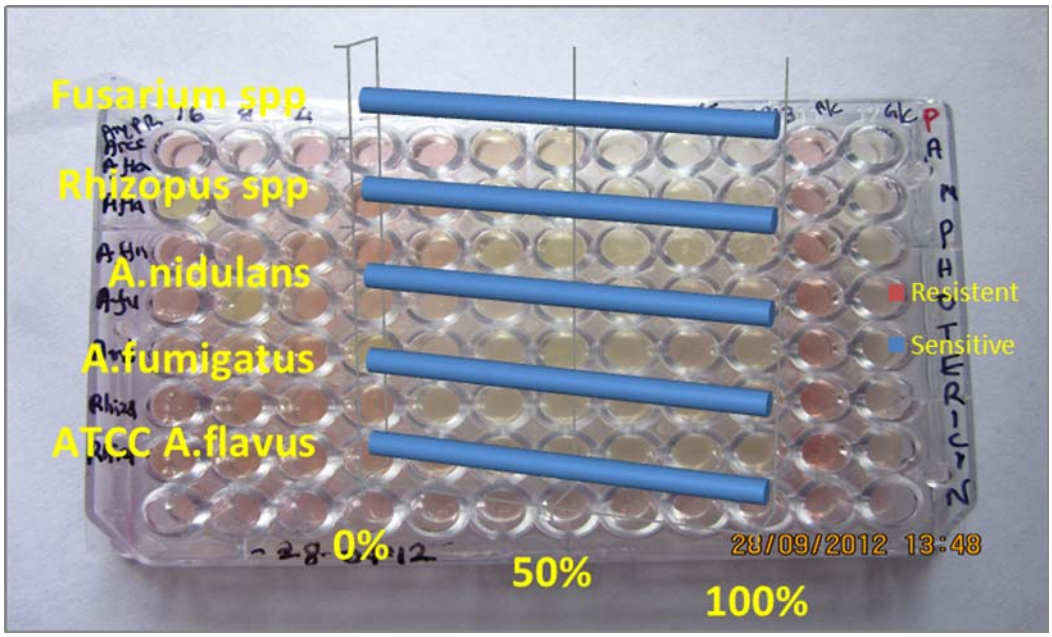


TABLE-17: ANTIFUNGAL SUSCEPTIBILITY PATTERN (MIC) OF AMPHOTERICIN B TO THE ISOLATED CANDIDA (n=17)

S.No	STRAIN	No.of.Isolates	Sensitive MIC≤1.0	Resistence MIC>1.0	Mean MICµg/ml
1.	Candida albicans ATCC® 90028	1	1(100%)	_	0.125
2	C.tropicalis	11	11(100%	_	0.125-0.5
3	C.parapsiosis	2	2(100%)	_	0.0625-0.25
4	C.glabrata	4	4(100%	_	0.125-0.5

All isolates were sensitive to amphotericin B.

Fig-17

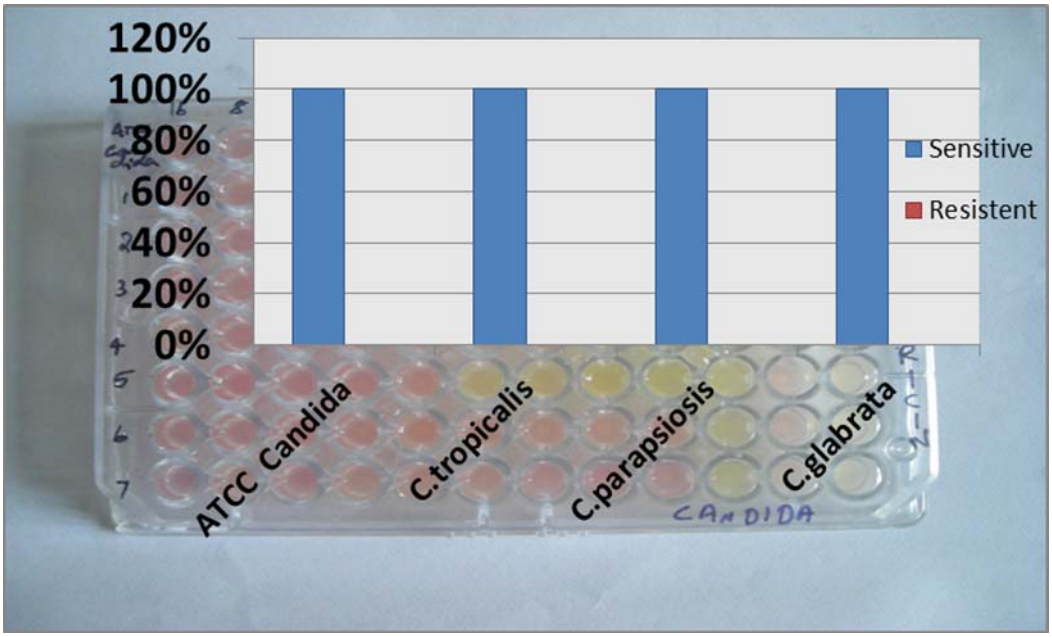


TABLE-18: ANTIFUNGAL SUSCEPTIBILITY PATTERN (MIC) OF FLUCONAZOLE TO THE ISOLATED CANDIDA (n=17)

S. No	STRAIN	No.of.Isolates	Sensitive MIC≤8	Resistence MIC>8	Mean MICµg/ml
1.	Candida albicans ATCC® 90028	1	1(100%)	_	4
2	C.tropicalis	11	11(100%	_	0.5-1.0
3	C.parapsiosis	2	2(100%)	_	1.0-2.0
4	C.glabrata	4	4(100%	_	0.5-1

All isolates were sensitive to fluconazole.

Fig-18

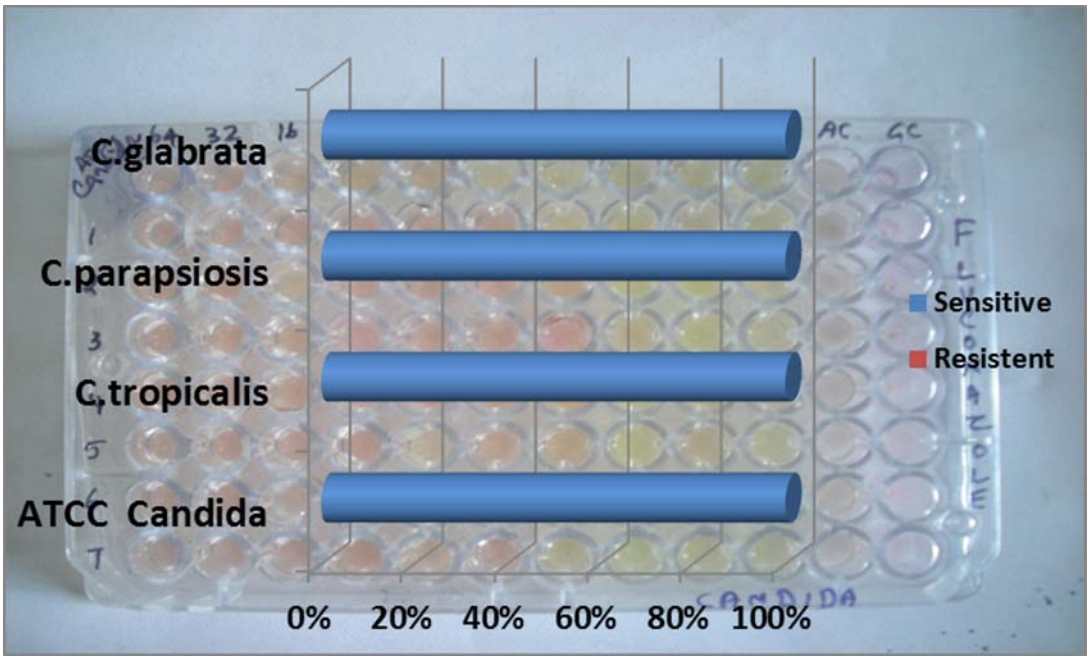


TABLE-19: ANTIFUNGAL SUSCEPTIBILITY PATTERN (MIC) OF ITRACONAZOLE TO THE ISOLATED CANDIDA (n=17)

S. No	STRAIN	No.of.Isolates	Sensitive MIC≤0.250	Resistence MIC>0.250	Mean MICµg/ml
1.	Candida albicans ATCC ®90028	1	1(100%)	_	0.250
2	C.tropicalis	11	11(100%	_	0.125-0.250
3	C.parapsiosis	2	2(100%)	_	0.125-0.250
4	C.glabrata	4	4(100%	_	0.0125-0.250

All isolates were sensitive to itraconzole.

Fig-19

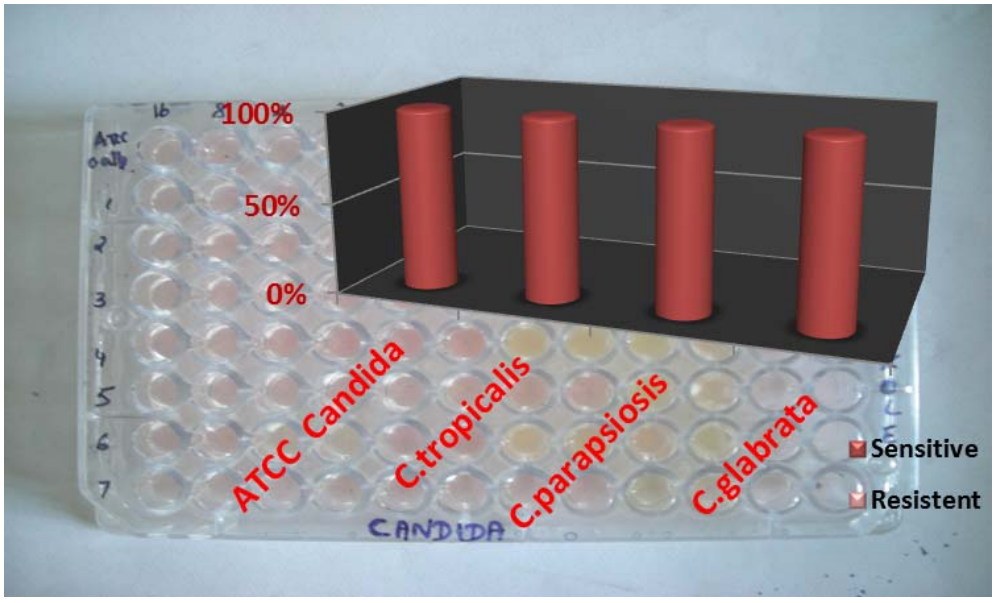
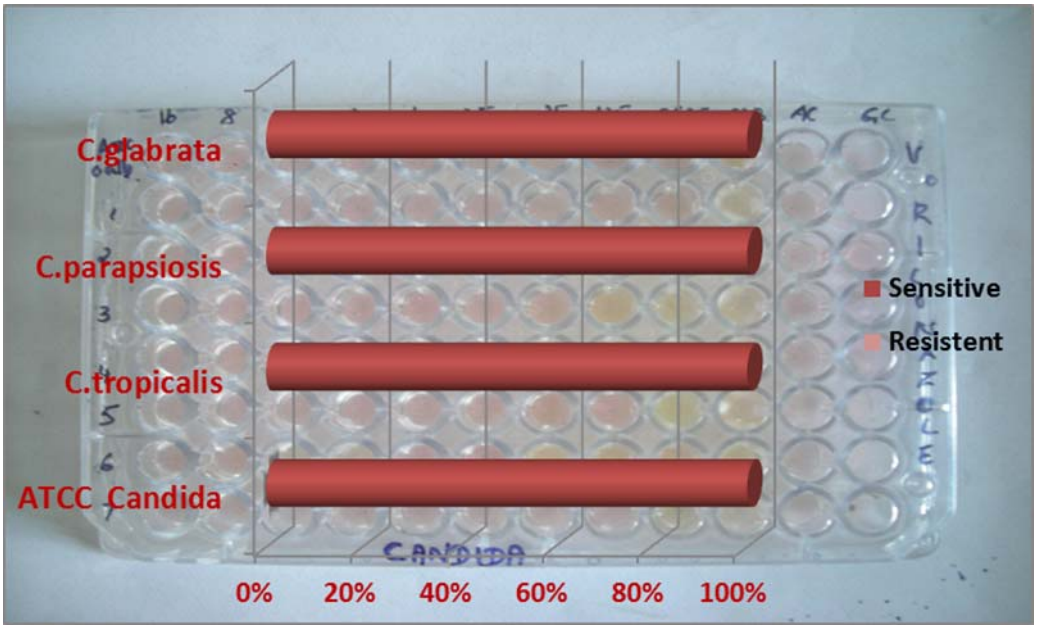


TABLE-20: ANTIFUNGAL SUSCEPTIBILITY PATTERN (MIC) OF VORICONAZOLE TO THE ISOLATED CANDIDA (n=17)

S.No	STRAIN	No.of.Isolates	Sensitive MIC≤1.0	Resistence MIC>1.0	Mean MICµg/ml
1.	Candida albicans ATCC® 90028	1	1(100%)	_	0.0625
2	C.tropicalis	11	11(100%	_	0.125-0.5
3	C.parapsiosis	2	2(100%)	_	0.0625-0.250
4	C.glabrata	4	4(100%	_	0.5-1

All isolates were sensitive to voriconazole.

Fig-20



DISCUSSION

A total of 150 diabetic patients who attended dermatology/ diabetology OPDs and was diagnosed as a case of superficial mycoses were studied during one year period (Oct 2011 to Sept 2012).

The age group of the patients were from 26 to 85, the mean age being 55.5years, [Table-1] same as that studied by Ditte Maria et al(2006) in 26-91yrs[62].The common age groups affected were between 41-60,which was similar to Blanka Havlickova et al (2008) who found in 44-57years age grours.[74,82]

Statitical analysis of the age factor (Fischer's exact test, p=0.8315) showed no significant difference in the occurrence of infection.

There were 78 males (52%) and 72 females (48%) [Table2] similar to M.Situm et al (1998) who found in 59% males and 41% females and also Kennedy et al (2007) who found in 53% and 47% respectively and stated that most studies in and around Chennai showed a male dominance[62,64,66,67,82].

The male to female ratio was 1.06:1. [Table-2] which correlated with that of Kennedy et al, Chennai, 1.12:1[77,86]. Heoprich et al (1994) has attributed the male preponderance to hormonal factor and P Veer (2007) et al to their more outdoor activities hence prone to trauma.

Statitical analysis of the gender (Fischer's exact test, p=0.1410) showed no significant difference in the occurrence of infection.

The distribution of type of diabetic patients in the study group were,typeI 12% and typeII 88% [Table3] ,similar to Eckhard et al(2007) who found in 18% and 82% respectively.[30,62,64] Statitical analysis of the type of diabetes (Fischer's exact test , p=0.6190) showed no significant difference in the occurrence of infection.

Out of the 150 diabetic patients studied 47 showed PPBS <160mg% (controlled DM) and 103 showed PPBS>160mg% (uncontrolled DM).The infection rate among the controlled was 59% and that in uncontrolled 43%. Statitical analysis (Fischer's exact test, p=0.08) showed no significant difference in the occurrence.Hence all diabetic patients are at the same risk of acquiring the infection.

Thus diabetic patients are susceptible to superficial mycoses irrespective of their age, gender, type of DM and blood sugar level ..[7,62]

A total of 63 skin and 87 nail samples were collected. [Table-4] as shown by Phoebe Rich et al that the onychomycosis was the most common infection among diabetic patients.[57,62,64,69].

Among the skin lesions,tinea corporis was the predominant [75,77-79,81,86] which occurred in 40(63.4%) patients followed by tinea cruris in 14(22.2%), tinea pedis 2(3.1%) and web space infection in3(4.7%). [Table-5]. Mixed infection were noted in 2 cases,comprising of tinea corporis and tinea manuum;tinea pedis and toe web space infectionsVerenkar MP et al(1991) has stated that the high incidence of tinea corporis and tinea cruris was probably

due to its symptomatic nature (pruritic) which leads the patient to seek medical advice.

Among nail infections distal and lateral subungual kertatosis was the predominant lesion which occurred in 58(66%) cases similar to Garg et al(2004) of 64.4% [Table-6].[68,82,85].

Out of 150 clinically diagnosed cases, direct microscopic examination(KOH) showed positivity in 35 skin lesions and in 30 nail samples .Thus the total KOHpositivity was 65(43%) [Table-7]. which was similar to JC Mohanty et al 43%(1999).[72]

The causal agents were isolated by culture in 39 skin lesions and in 34 nail samples. [Table-8]. Thus the total culture positivity was 73(48%) which only slightly varied with Pankajalakshmi et al, Chennai(1981) 44%,Sharma N L et al,Shimla(1987)45% and Mallick AK et al,Rohtak (1996) 53%.

Thus a total of 35(23.3%) samples were positive on direct examination and culture which was similar to Mallick AK et al 23%(1996) and slightly lower than Vasu BH et al,Warangal(1966) 26%.[Table-9]

Samples negative by both the techniques was 51(34%).Samples positive by direct examination and negative on culture was 28(18.6%). Further 36 (24%) sample isolated on culture were negative on direct examination [Table-9] which was slightly less than JC Mohanty et al 30% (1997). (p=0.4175; Fischer exact test, no statistical significant difference in the diagnostic efficacy)

In skin lesions, dermatophyte was the major pathogen isolated which accounted for 33(84%) out of culture positive cases followed by candida species 6(15%) [Table-10] as shown by A Lugo.Somolinos et al that 15% of isolates in diabetes were candida. [62, 75.77, 81, 93]. Among the candida species the predominant species isolated from skin was Candida tropicalis, 4(66%) [Table-11].

Among the dermatophytes,Trichophyton mentagrophytes 21(63%) was the commonest strain followed by Trichophyton rubrum, 9(27%) [Table-11], similar to M.Situm et al (1998) and Romano C et al (2001studies) [26,74]

In nail infection, dermatophyte was the major pathogen which accounted for 14(41%) out of the culture positive cases followed by candida 11(32%) and nondermatophyte mould 9(26%) [Table-11]

Among the dermatophytes isolated in nail samples, Trichophyton mentagrophytes 12(85%) was the commonest followed by Trichophyton rubrum (14%) [Table-11] similar to Macura AB et al (2007) [26]. The candida species isolated in nail was also predominantly Candida tropicalis 7(63%). [Table-11].

The predominant variant was Trichophyton mentagrophytes var inter digitale (anthropophilic) and two isolates were Trichophyton mentagrophytes var mentagrophytes (Zoophilic).

The total nondermatophytes isolated in nail lesions (repeat isolate) was 9(26%), [Table-11], namely Rhizopus spp, Fusarium spp and Aspergillus spp [66,70,95,96,98]. As Wg Cdr Grover S et al (2003) stated that NDM colonises the damaged tissues and can cause infection in diabetic patients and moreover Mohammad Rahbar et al (2010) explained that the spores of NDM which are

ubiquitous in the environment can transiently colonise healthy skin and causes debilitating disease in immunocompromised patients.

Infection with two strains were noted in one case comprising of Trichophyton mentagrophytes and Candida parapsilosis of toe nail similar to that showed by Mohammad Rahbar et al(2008). [26,97,98].

The antifungal susceptibility pattern (MIC),of the isolates were studied. Among dermatophytes ,all isolates were sensitive to terbinafine,itraconazole and ketoconazole except two strains . [Table-12-14] Out of the resistant strains ,one (T.rubrum) was resistant to all the three drugs and the other (T.rubrum) to terbinafine. [89,97,99].

Lisa Matricciani et al (2011) has contributed hyperglycaemia as the reason for drug resistance.In this study the multidrug resistant patient had PPBS of 425mg%.

Colin.S.osborne et al (2005) has found that some strains of T.rubrum isolated from diabetic patient with onychomycosis showed intrinsic resistance to terbinafine which on prolonged exposure to the drug can raise the MIC values.Similar findings were shown by Pranab K.Mukherjee et al (2003).

Cervelatti EP et al (2006) and Fachin AL et al(2006) has detailed the involvement of ABC transporter gene in the development of resistance to azoles inT.rubrum.

The candida species (17) which were tested against amphotericin B, itraconazole and voriconazole, fluconazole [Table-17-20] and the nondermatophyte moulds (10) to amphotericin B, itraconazole and voriconazole showed 100% sensitivity pattern. [Table-14-16].

SUMMARY

Out of the 150 diabetic patient with superficial mycoses studied,

- Male to female ratio was 1.06:1.
- The commonest age group affected were 41-50 (34%) followed by 51-60(25%).
- It was more common in type II than in type I diabetes mellitus.
- It occured in all diabetic patients irrespective of their age, gender,type of DM and blood glucose levels.
- The commonest skin lesion was tinea corporis 40(63%) followed by tinea cruris 14(22%).
- The commonest nail lesion was distal and lateral subungual onychomycosis (DLSO) 58(66%).
- The direct microscopy (KOH) was positive in 65(43%) and culture in 73(48%) of clinically diagnosed cases.
- Isolation rate in culture medias namely SDA and DTM were the same but earliest in DTM (5-7days).
- The commonest isolates in skin were dermatophytes 33(84%) followed by candida 6(15%) and in nails,dermatophytes 14(41%),candida 11(32%) and nondermatophyte 9(12%).

- Among the total dermatophytes isolated, T.mentagrophytes 33(45%) was the commonest pathogen and in Candida species,C.tropicalis 11(15%) .

- Among the total dermatophytes isolated, two isolates showed resistance to antifungal agents.One was to all the three drugs(Terbinafine,ketoconazole and itraconazole) and other to terbinafine alone.

- All the candida species showed 100% sensitivity to amphotericin B,Itraconazole,Fluconazole and voriconazole.

- Nondermatophyte moulds also showed 100% sensitivity to amphotericin B,Itraconazole, and voriconazole.

CONCLUSION

Diabetes is increasing worldwide and the mortality due to diabetic foot syndrome is also on the rise.Superficial mycoses accounts the common cutaneous manifestation and especially onychomycosis which can act as a chronic reservoir. Due to associated complications like neurovasculopathy, obesity and hyperglycaemia,the tissues are further damaged before the patient can recognise the lesion.The chronicity and tissue disruption can lead to secondary bacterial infection.

Clinical suspicion, early laboratory examination to confirm diagnosis and appropriate treatment is very crucial in this group of patients.

Diabetic patients are susceptible to superficial mycoses irrespective of their age, gender, type of DM and blood sugar level as proved with statistical analysis.Thus their immunosuppressive state is the main risk factor.

Direct microscopic examination and culture identification plays the major part in the management.Though Sabouraud dextrose agar with antimicrobials which needs incubation for 4-8weeks,is the commonly used media for isolation, Dermatophyte test medium which give presumptive identication within a week can be used instead in these group of patients.

Apart from dermatophytes which is the commonest isolates in superficial mycoses, other agents like Candida and non dermatophyte moulds which were considered as contaminants or coloniser is also emerging as a

pathogen especially in these group of patients.Hence repeated isolation will prove its pathogenicity.

Though early complete course of treatment gives mycological cure, resistant strains are also occurring which can act as a chronic reservoir of infection.Hence routine antifungal susceptibility testing especially in these group of patients for whom mycological cure can save a limb has to be done routinely for timely interventions.

LIST OF ABBREVIATIONS

AFST	:	Antifungal susceptibility testing
ATCC	:	American type culture collection
A.f	:	Aspergillus fumigatus
A.n	:	Aspergillus nidulans
CLSI	:	Clinical and laboratory standard institute
C.g	:	Candida glabrata
CMA	:	Corn meal agar
C.p	:	Candida parapsilosis
C.t	:	Candida tropicalis
DM	:	Diabetes mellitus
DMSO	:	Dimethyl sulphoxide
DLSO	:	Distal and lateral subungual onychomycosis
DTM	:	Dermatophyte test medium
Fus	:	Fusarium species
GT	:	Germ tube
KOH	:	Potassium hydroxide

LPCB	:	Lactophenol cotton blue
MIC	:	Minimum inhibitory concentration
NDM	:	Non dermatophyte mould
PPBS	:	Post prandial blood sugar
PDA	:	Potato dextrose agar
PSO	:	Proximal Subungual Onychomycosis
Rhi	:	Rhizopus species
RPMI	:	Rose Parkwell memorial institute.
SDA	:	Sabouraud’s dextrose agar
SAP	:	Secretory aspartyl proteases
TDO	:	Total Dystrophic Onychomycosis
T.m	:	Trichophyton mentagrophytes
T.r	:	Trichophyton rubrum
T.t	:	Trichophyton tonsurans
T.v	:	Trichophyton verrucosum
WSO	:	White Superficial Onychomycosis

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www.Sigmaaldrich.com/preparation of RPMI1640 medium.

PROFORMA

NAME: AGE/SEX: OP/IP:

OCCUPATION:

PRESENT COMPLAINTS:

SITE OF LESION:

PRIMARY/RELAPSE:

PET ANIMALS:

TYPE OF DM:

DURATION OF DM:

B.SUGAR:

TREATMENT HISTORY:

FAMILY H/O DM & LESIONS

SAMPLE:SKIN/NAIL/HAIR

LAB INVESTIGATION:

KOH MOUNT:

CULTURE:SDA

DTM

GRAM STAIN:

LPCB MOUNT:

SLIDE CULTURE:

UREA HYDROLISIS TEST

IN VITRO HAIR PERFORATION TEST:

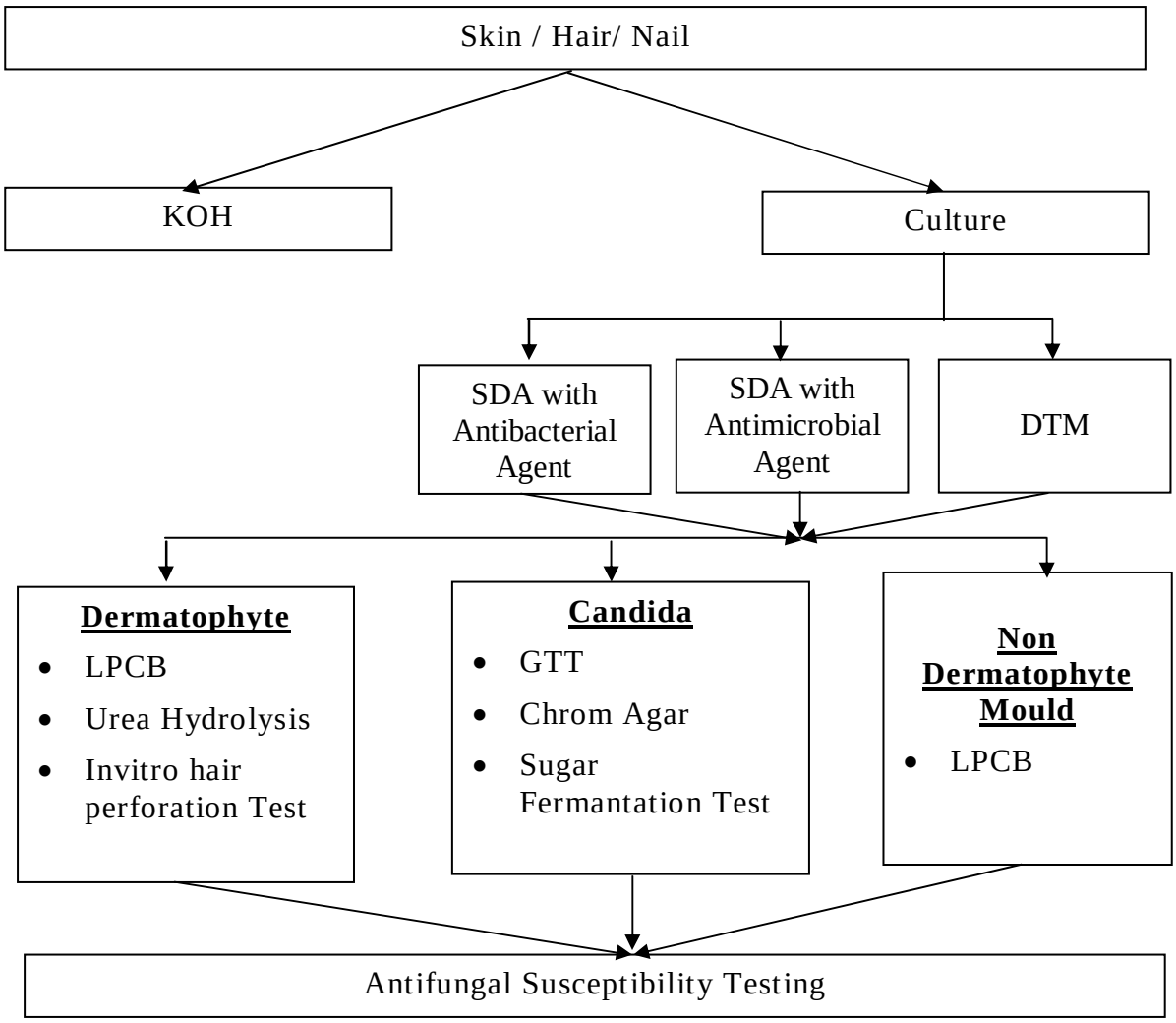
GERM TUBE TEST:

CHROM AGAR:

FERMAENTATION OF SUGAR

INTERPRETATIONS:

FLOW CHART


Skin / Hair/ Nail
KOH
Culture
SDA with Antibacterial Agent
SDA with Antimicrobial Agent
DTM
Dermatophyte
LPCB
Urea Hydrolysis
Invitro hair perforation Test
Candida
GTT
Chrom Agar
Sugar Fermantation Test
Non Dermatophyte Mould
LPCB
Antifungal Susceptibility Testing

ANNEXURE

SDA WITH ANTIBIOTICS [20 PG 509]

Ingredients:

Peptone-10gm

Dextrose-40gm

Agar-20gm

Cycloheximide-500mg

Chloramphenicol-50mg

Gentamicin-20mg

Distilled water-1000ml

All the above mentioned ingredients are autoclaved and adjust pH at 5.6. Dissolve cycloheximide in 10ml acetone and similarly dissolve chloramphenicol/gentamicin in 10ml of 95% alcohol and added to the boiling medium.Dispense in tubes ,allowed to cool in slanted position. Store at 4^0 C. shelf life of 30 days in test tube and 14 days in petridishes.

DTM [20 Pg 510]

Phyton-10gm

Dextrose-10gm

Phenol red solution-40ml

8N HCl-6ml

Actidione-500mg

Gentamicin-100mg

Agar-20gm

Distilled water-1000ml

Final pH-5.5+/-0.4

The phenol red solution is 0.5 gm in 15ml of 1N NaOH made upto 100ml with distilled water. Adjust pH to 5.5.

GRAM STAIN [60]

Crystal violet (0.5-2%)-10 gm

Absolute alcohol (100% ethanol)-100 ml

Distilled water-1000 ml

Dissolve the dye in the alcohol,filter through filter paper and add to th water.

Iodine solution☹Lugol's)

Iodine-10 gm

Potassium iodide -20 gm

Distilled water-1L

Add potassium iodide in 250 ml water followed sby 10 gm iodine. When iodine is dissolved,make up to 1 litre with water.

Dilute carbol fuchsin:

Basic fuchsin -5 gm

Phenol-25 gm

Alcohol (95% or 100%,ethanol)-50 ml

Distilled water-500 ml

Dissolve the fuchsin in the phenol by placing them in a 1litre flask over a boiling water bath for about 5min,shaking the contents from time to time. When solution is complete, add the alcohol and mix thoroughly. Then add distilled water. Filter the mixture before use.

This Ziehl Neelsen's stain is diluted 10-20 times its volume of water.

LPCB **MOUNT**:[20 Pg 515]

Ingredients:

Melted Phenol-20ml

Lactic acid-20ml

Glycerol-40ml

Cotton blue-0.05gm

Distilled water-20ml

Mix all ingredients properly and dissolve 0.05 gm of Cotton blue stain in distilled water before mixing with remaining reagents. The phenol acts as disinfectant. Lactic acid preserves morphology of fungi and glycerol is a hygroscopic agent which prevents drying. The cotton blue stains outer wall of fungus.

KOH MOUNT[20 Pg516]

Ingredients:

Potassium hydroxide-10gm

Glycerol-10ml

Distilled water-80ml

To solution of 10% KOH, 10ml glycerol is added to prevent drying. Mix these ingredients properly and store the solution at room temperature.

NOTE:For 20% KOH ,20gm Potassium hydroxide is used.

CHROM AGAR:[60]

Ingredients:

Glucose-20 gm

Agar-15 gm

Peptone-10 gm

Chromogenic mix-2 gm

Chloramphenicol-0.5 gm.

Mix all ingredients thoroughly in distilled water and make it to final volume of 1litre. Gently heat in a boiling water bath to dissolve components. After cooling to 45-50^0 C the media is poured into sterile petri dishes.

FERMENTATION OF SUGAR [20,44]

Peptone -10 gm

Andrade's indicator(0.005%)-10 ml

Sugar (Glucose,Lactose.Sucrose,Galactose,Maltose,Trehalose)-20 gm

NaCl -0.5%

Distilled water-1000 ml.

Dissolve the peptone and Andrade's indicator in 1litre of water and add 20 gm of the sugar. Distribute in 3ml amounts in standard test tubes containing an inverted Durham tube. Sterilize by inspissation.

10% YEAST EXTRACT [43]

Yeast extract -10 gm

Distilled water-100ml

Mix the yeast extract and distilled water in a flask and swirl to dissolve. Filter sterilize the solution and store it in sterile flask in a refridgerator until it is used.

MODIFIED CHRISTENSEN'S MEDIUM FOR UREA HYDROLYSIS [20]

Peptone-1 gm

Sodium chloride-5 gm

Disodium phosphate-1.2 gm

Monopotassium phosphate-0.8 gm

Phenol red -0.012 gm

Dextrose-1.5 gm

Agar -15 gm

Distilled water-1000 ml.

Urea,20%,solution,sterile-100ml

After dissolution of the above ingredients by heat, 5 ml of phenol red solution (0.2% in 50 % alcohol) was added after which autoclaving was done at 115^0 C for 15 mins. On cooling to 50^0 C, 100 ml of urea (20% aqueous solution, sterilized by filtration) was added. The medium was poured into slopes with the butt 1" deep and the slant 1.5" long.

RPMI 1640(ROSEWELL PARK MEMORIAL INSTITUTE [100]

RPMI 1640 is available in powder (without L-glutamine,L-Leucine,L-Lysine,L-Methionine and sodium bicarbonate)

PROCEDURE

To the 90% of the required final volume of distilled water, the RPMI 1640 powder is added and gently stirred.

Once completely dissolved the pH is adjusted to 7.2 with 1NNaOH.

To the dissolved solution 2.0g/litre of sodium bicarbonate is added and stirred completely.

The pH is adjusted 0.1-0.3 below the desired pH with 1NHCl/1NNaOH.

Remaining 10% of the distilled water is also added to make the final volume.

The solution is sterilized immediately by filtering through membrane of porosity 0.22µ.

MASTER CHART

S.No	Age	Sex	DM	PPBS	Lesion	KOH	CULTURE	LPCB/GRAM	UREASE	HAIR PERFORATION	AMPHO	VORI	FLUCON	ITRA	TERBINA	KETO
1	37	F	2	150	DLSO	P	P	T.Menta	P	P				S	S	S
2	47	M	2	172	T.corporis	N	N	NG								
3	38	F	2	454	T.cruris	N	P	C.parapsil			S	S	S	S		
4	38	F	2	243	TDO	P	N	NG								
5	26	F	1	188	web space	N	P	C.trpicalis			S	S	S	S		
6	47	F	2	208	PSO	P	P	TMENTA	P	P				S	S	S
7	47	F	2	132	web space	N	P	C.glabrata			S	S	S	S		
8	62	F	2	149	WSO	N	P	TRUBRUM						S	R	S
9	48	F	2	202	TDO	P	N	NG								
10	42	F	2	112	T.corporis	P	P	T.Menta	P	P				S	S	S
11	46	F	2	140	T.corporis	N	N	NG								
12	50	F	1	169	DLSO	P	N	NG								
13	70	M	2	206	T.corporis	P	P	T.Menta	P	P				S	S	S
14	50	F	2	144	DLSO	N	N	NG								
15	85	M	2	234	T.corporis	N	N	NG								
16	40	F	2	190	T.corporis	P	P	T.TONS						S	S	S
17	75	M	2	156	T.corporis	N	P	T.Menta	P	P				S	S	S
18	50	F	2	144	DLSO	P	P	TRUBRUM						S	S	S
19	50	M	2	120	WSO	N	P	T.Menta	P	P				S	S	S
20	47	M	2	177	DLSO	N	N	NG								
21	56	M	2	196	web space	N	N	NG								
22	67	F	2	184	T.corporis	N	N	NG								
23	58	M	1	220	WSO	P	P	C.tropica			S	S	S	S		
24	50	F	2	144	DLSO	N	P	C.tropica			S	S	S	S		
25	56	M	2	194	PSO	N	P	Rhizopus			S	S		S		
26	32	M	2	204	DLSO	P	N	NG								
27	55	M	2	223	T.manum	N	P	T.Menta	P	P				S	S	S
28	54	F	2	320	WSO	P	P	CPARA			S	S	S	S		
29	73	M	2	174	T.corporis	N	N	NG								
30	55	M	2	113	T.corporis	N	P	T.Menta	P	P				S	S	S
31	77	F	1	404	TDO	P	N	NG								
32	50	M	2	114	T.cruris	P	P	T.Menta	P	P				S	S	S
33	50	M	2	256	T.corporis	P	N	NG								
34	54	F	2	330	WSO	N	P	C.topicalis			S	S	S	S		
35	65	F	2	104	DLSO	N	N	NG								
36	50	M	2	114	T.corporis	N	P	T.Menta	P	P				S	S	S
37	50	M	2	245	PSO	N	N	NG								
38	40	F	2	425	T.cruris	N	N	NG								
39	42	F	2	114	DLSO	P	P	TMENTA	P	P				S	S	S
40	45	M	2	305	T.corporis	P	P	T.rubrum						S	S	S

S.No	Age	Sex	DM	PPBS	Lesion	KOH	CULTURE	LPCB/GRAM	UREASE	HAIR PERFORATION	AMPHO	VORI	FLUCON	ITRA	TERBINA	KETO
41	44	M	2	425	T.cruris	N	P	T.rubrum						S	S	S
42	45	M	2	323	DLSO	N	N	NG								
43	74	M	2	425	WSO	N	N	NG								
44	54	F	1	235	DLSO	N	P	C.glabrata			S	S	S	S		
45	56	F	2	204	DLSO	P	N	NG								
46	60	F	2	245	T.corporis	P	P	T.Menta	P	P				S	S	S
47	38	M	2	123	DLSO	N	P	c.Tropicalis			S	S	S	S		
48	32	M	2	119	WSO	N	N	NG								
49	64	F	2	107	DLSO	P	N	NG								
50	55	F	2	292	T.corporis	P	P	T.Menta	P	P				S	S	S
51	38	M	1	235	TDO	P	N	NG								
52	54	M	2	196	WSO	N	N	NG								
53	59	M	2	198	DLSO	N	N	NG								
54	59	M	2	256	DLSO	N	P	TMENTA	P	P				S	S	S
55	44	M	2	241	WSO	P	N	NG								
56	65	F	2	120	T.corporis	P	P	T.rubrum						S	S	S
57	35	F	1	240	T.corporis	N	P	T.verruco						S	S	S
58	54	F	2	147	TDO	P	N	NG								
59	42	F	2	260	T.cruris	P	P	T.Menta	P	P				S	S	S
60	65	F	2	208	T.cruris	P	P	T.verruco						S	S	S
61	61	F	2	237	DLSO	N	P	T.Menta	P	P				S	S	S
62	36	M	1	177	T.corporis	N	N	NG								
63	45	M	2	235	T.cruris	N	N	NG								
64	41	F	2	212	T.corporis	N	N	NG								
65	38	F	1	180	DLSO	N	N	NG								
66	75	M	2	270	DLSO	P	N	NG								
67	73	M	2	126	DLSO	P	N	NG								
68	45	M	2	260	WSO	P	N	NG								
69	41	M	2	245	DLSO	N	N	NG								
70	75	M	2	112	DLSO	N	P	Fusarium			S	S		S		
71	57	M	2	354	DLSO	P	P	TMENTA	P	P				S	S	S
72	55	M	2	187	DLSO	N	P	Rhizopus			S	S		S		
73	52	M	2	268	DLSO	P	N	NG								
74	61	F	2	245	DLSO	P	P	CTROPI			S	S	S	S		
75	54	M	2	129	T.corporis	P	P	T.Menta	P	P				S	S	S
76	37	F	2	208	DLSO	P	P	T.Menta	P	P				S	S	S
77	47	M	2	132	T.corporis	P	N	NG								
78	38	F	2	149	T.cruris	N	P	C.TROPIC			S	S	S	S		
79	38	F	2	202	DLSO	N	N	NG								
80	26	F	1	120	DLSO	P	N	NG								
81	47	F	2	240	DLSO	P	P	A.FUMI			S	S		S		
82	47	F	2	147	WSO	P	P	C.glabrata			S	S	S	S		
83	62	F	2	150	T.corporis	N	N	NG								

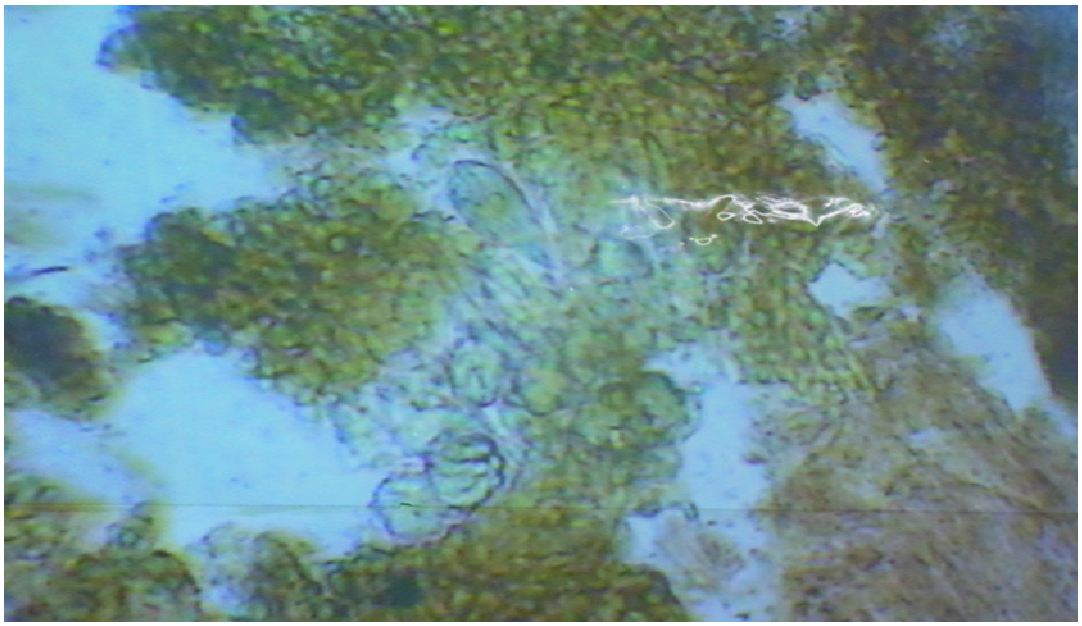
S.No	Age	Sex	DM	PPBS	Lesion	KOH	CULTURE	LPCB/GRAM	UREASE	HAIR PERFORATION	AMPHO	VORI	FLUCON	ITRA	TERBINA	KETO
84	48	F	2	172	DLSO	N	N	NG								
85	42	F	2	454	DLSO	P	N	NG								
86	46	F	2	243	T.corporis	P	P	T.Menta	P	P				S	S	S
87	50	F	1	188	T.cruris	P	P	T.rubrum						S	S	S
88	70	M	2	208	DLSO	N	N	NG								
89	50	F	2	132	T.corporis	N	N	NG								
90	85	M	2	330	T.corporis	N	N	NG								
91	40	F	2	174	DLSO	P	N	NG								
92	75	M	2	113	WSO	N	P	T.Menta	P	P				S	S	S
93	50	F	2	177	T.corporis	P	P	T.Menta	P	P				S	S	S
94	50	M	2	235	WSO	N	N	NG								
95	47	M	2	212	T.cruris	P	N	NG								
96	56	M	2	180	T.corporis	P	P	T.Menta	P	P				S	S	S
97	67	F	2	196	T.corporis	N	P	T.Menta	P	P				S	S	S
98	58	M	1	198	DLSO	P	P	Fusarium			S	S		S		
99	50	F	2	256	WSO	P	P	A.nidulan			S	S		S		
100	56	M	2	241	DLSO	N	P	T.Menta	P	P				S	S	S
101	32	M	2	120	DLSO	P	N	NG								
102	55	M	2	240	T.manum	P	P	C.TROPI			S	S	S	S		
103	54	F	2	147	T.corporis	N	N	NG								
104	73	M	2	260	T.corporis	N	N	NG								
105	55	M	2	243	WSO	N	P	T.Menta	P	P				S	S	S
106	77	F	1	188	T.cruris	P	P	T.rubrum						S	S	S
107	50	M	2	208	DLSO	N	P	T.Menta	P	P				S	S	S
108	50	M	2	132	DLSO	N	P	C.TROPI			S	S	S	S		
109	54	F	2	132	WSO	N	P	Rhizopus			S	S		S		
110	65	F	2	149	DLSO	P	N	NG								
111	50	M	2	202	DLSO	P	P	Rhizopus			S	S		S		
112	50	M	2	270	DLSO	N	N	NG								
113	40	F	2	126	T.corporis	N	P	T.Menta	P	P				S	S	S
114	42	F	2	260	WSO	N	N	NG								
115	45	M	2	245	T.corporis	N	N	NG								
116	44	M	2	112	T.facei	N	P	T.rubrum						S	S	S
117	45	M	2	174	DLSO	N	N	NG								
118	74	M	2	113	T.corporis	P	P	T.Menta	P	P				S	S	S
119	54	F	1	234	T.cruris	N	P	C.TROPI			S	S	S	S		
120	56	F	2	190	DLSO	P	N	NG								
121	60	F	2	156	T.barbae	N	P	T.Menta	P	P				S	S	S
122	38	M	2	144	DLSO	P	N	NG								
123	32	M	2	120	T.corporis	P	P	T.Menta	P	P				S	S	S
124	64	F	2	177	DLSO	P	N	NG								
125	55	F	2	243	T.pedis	P	P	T.Menta	P	P				S	S	S
126	38	M	1	188	T.corporis	N	P	T.rubrum						S	S	S

S.No	Age	Sex	DM	PPBS	Lesion	KOH	CULTURE	LPCB/GRAM	UREASE	HAIR PERFORATION	AMPHO	VORI	FLUCON	ITRA	TERBINA	KETO
127	54	M	2	208	DLSO	N	N	NG								
128	59	M	2	132	DLSO	P	N	NG								
129	59	M	2	425	WSO	N	P	C.glabrata			S	S	S	S		
130	44	M	2	323	DLSO	N	N	NG								
131	65	F	2	425	T.pedis	P	P	TRUBRUM						R	R	R
132	35	F	1	235	DLSO	N	P	C.TROP			S	S	S	S		
133	54	F	2	243	DLSO	N	N	NG								
134	42	F	2	188	WSO	P	N	NG								
135	65	F	2	208	DLSO	N	N	NG								
136	61	F	2	132	DLSO	N	P	A.FUMI			S	S		S		
137	36	M	1	204	DLSO	N	N	NG								
138	45	M	2	223	WSO	N	N	NG								
139	41	F	2	320	DLSO	N	N	NG								
140	38	F	1	174	DLSO	N	N	NG								
141	75	M	2	113	T.corporis	N	N	NG								
142	73	M	2	404	T.corporis	P	P	T.rubrum						S	S	S
143	45	M	2	196	DLSO	N	N	NG								
144	41	M	2	198	T.cruris	P	N	NG								
145	75	M	2	245	T.corporis	N	N	NG								
146	57	M	2	123	DLSO	N	N	NG								
147	55	M	2	119	T.cruris	P	P	T.Menta	P	P				S	S	S
148	52	M	2	107	WSO	N	N	NG								
149	61	F	2	292	T.corporis	N	N	NG								
150	54	M	2	235	T.corporis	P	N	NG								

M-MALE
F-FEMALE
PPBS-POSTPRANDIAL BLOOD SUGAR
P-POSITIVE
N-NEGATIVE
NG-NO GROWTH
S-SENSITIVE
R-RESISTANCE

Image-1 TINEA CORPORIS DLSO

Image-2 KOH MOUNT-PLENTY OF ARTHROSPORES

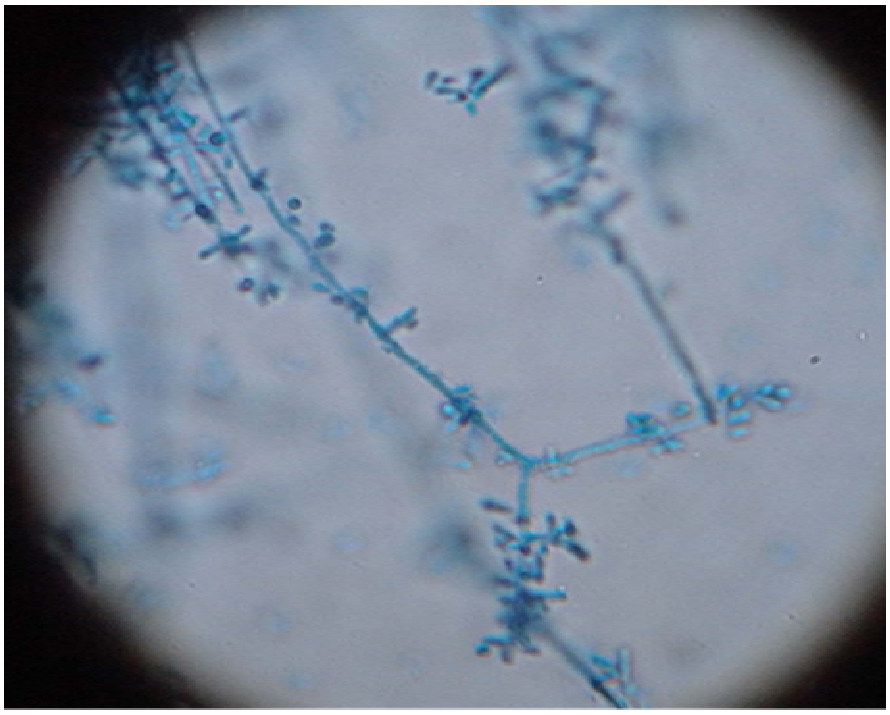


TRICHOPHYTON MENTAGROPHYTES VAR MENTAGROPHYTES MACROSCOPY

MICROSCOPY

TRICHOPHYTON MENTAGROPHYTES VAR INTERDIGITALE, MACROSCOPY

MICROSCOPY

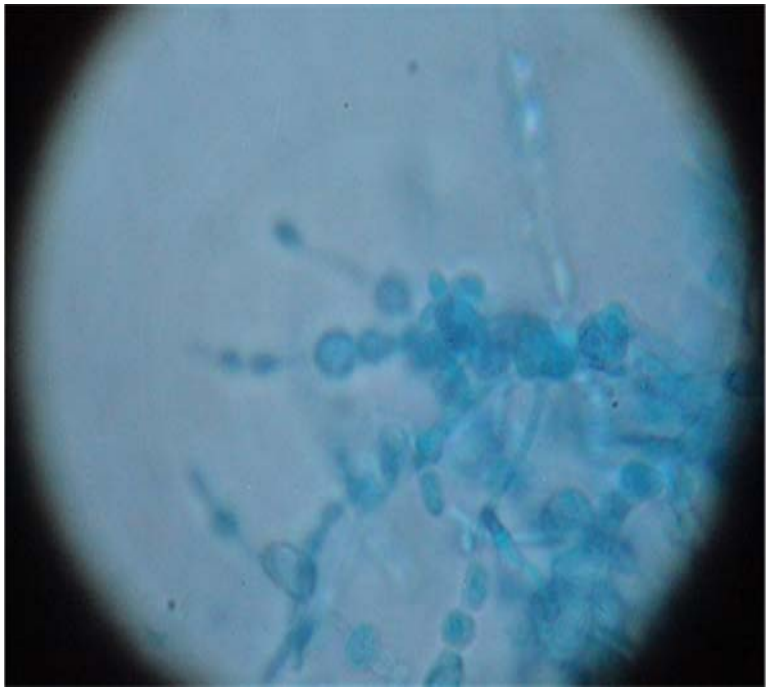


TRICHOPHYTON RUBRUM, MACROSCOPY

Microscopy

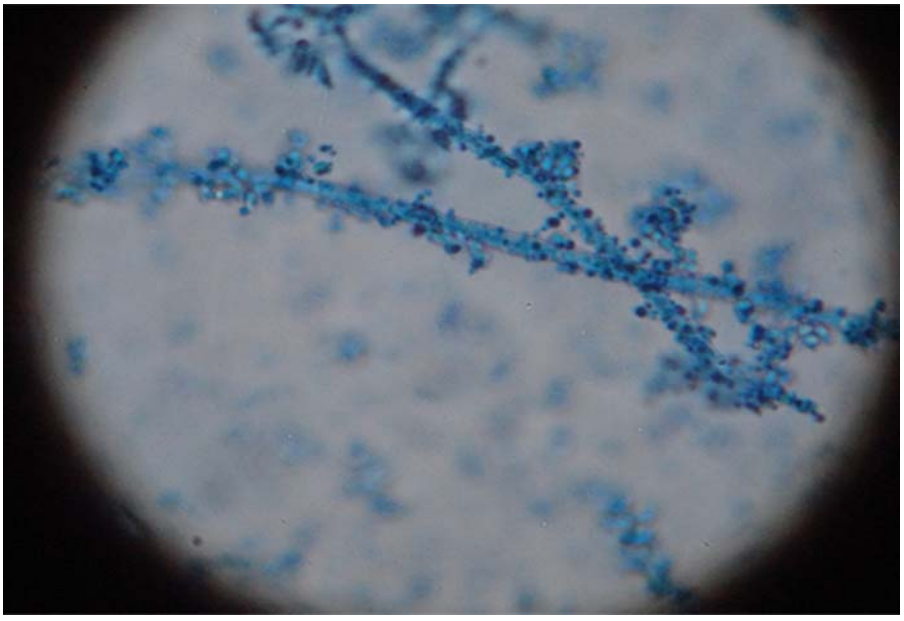
T.VERRUCOSUM MACROSCOPY

MICROSCOPY

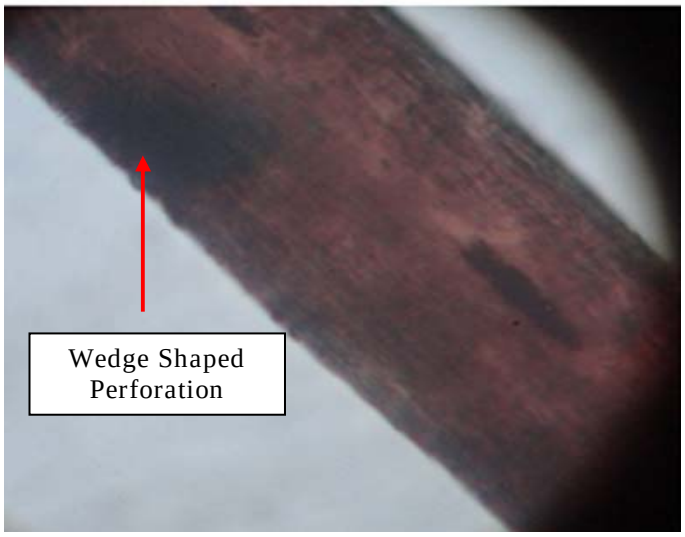


TRICHOPHYTON TONSURANS MACROSCOPY

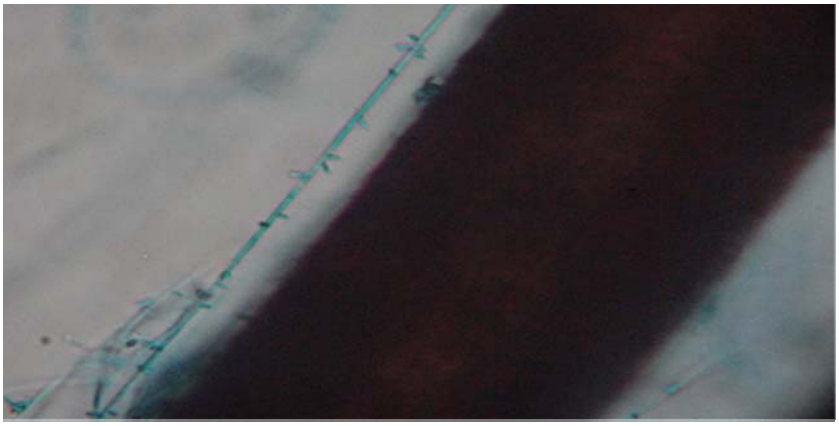
MICROSCOPY



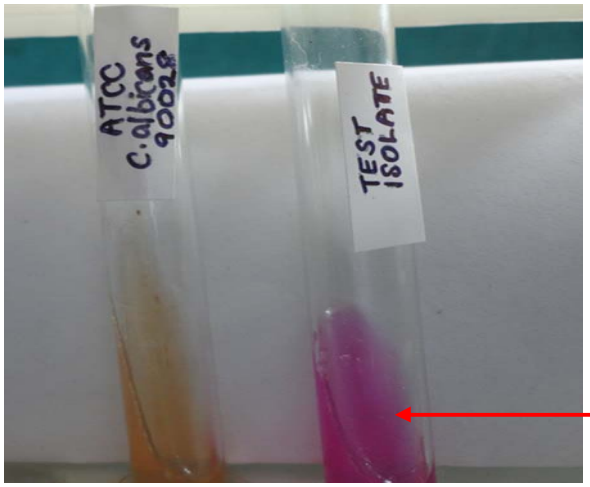
IN VITRO HAIR PERFORATION TEST-POSITIVE



IN VITRO HAIR PERFORATION TEST- NEGATIVE



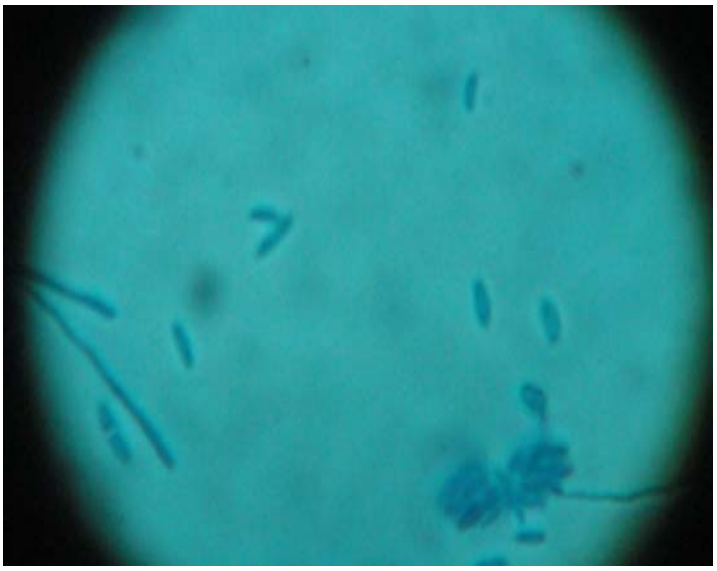
UREA HYDROLYSIS



Urease
Positive

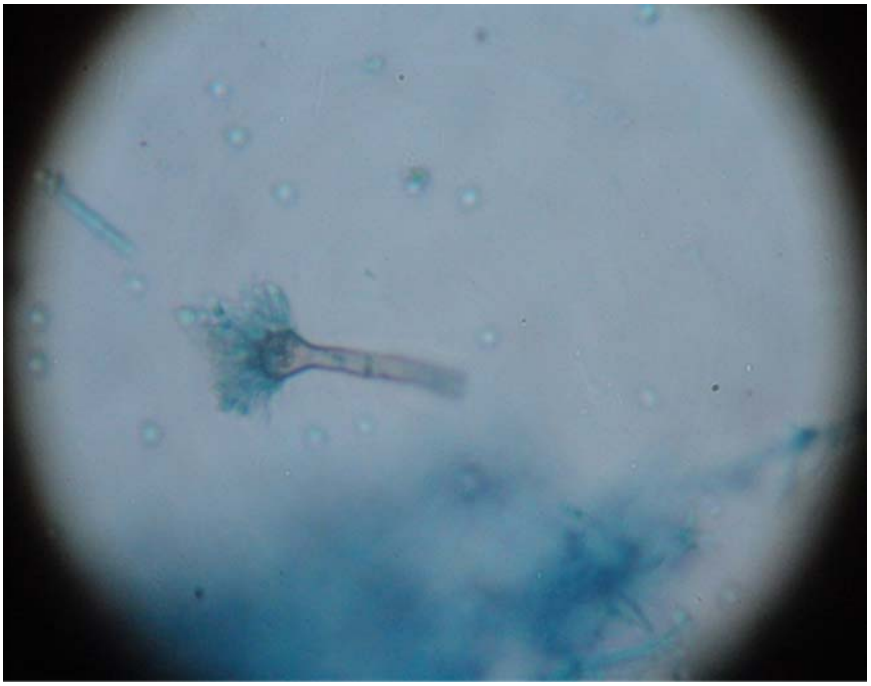
FUSARIUM SPECIES MACROSCOPY

MICROSCOPY



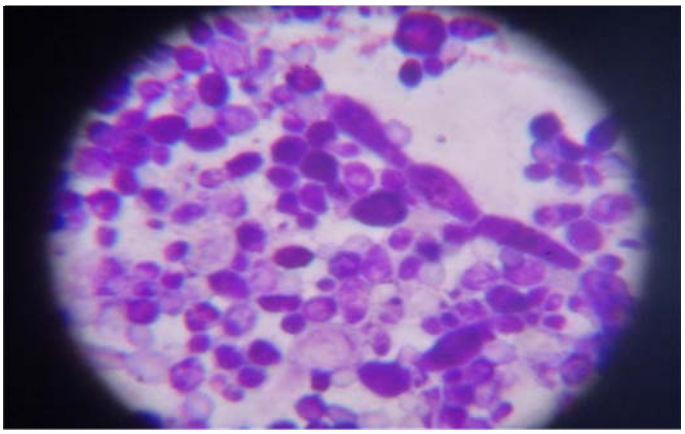
ASPERGILLUS NIDULANS MACROSCOPY

MICROSCOPY

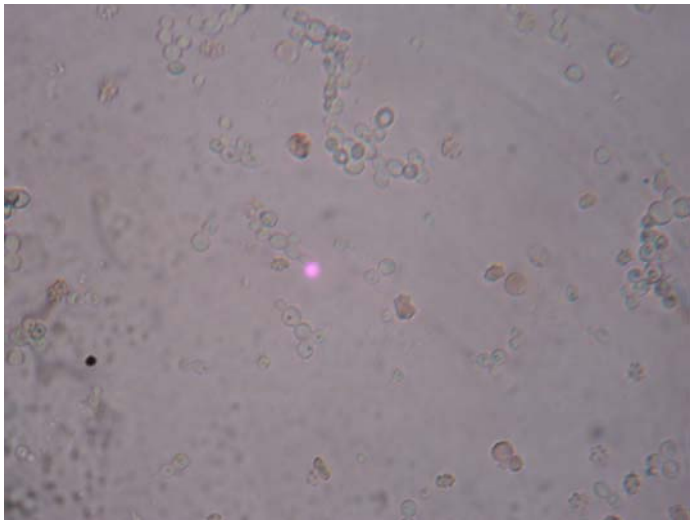


CANDIDA TROPICALIS MACROSCOPY

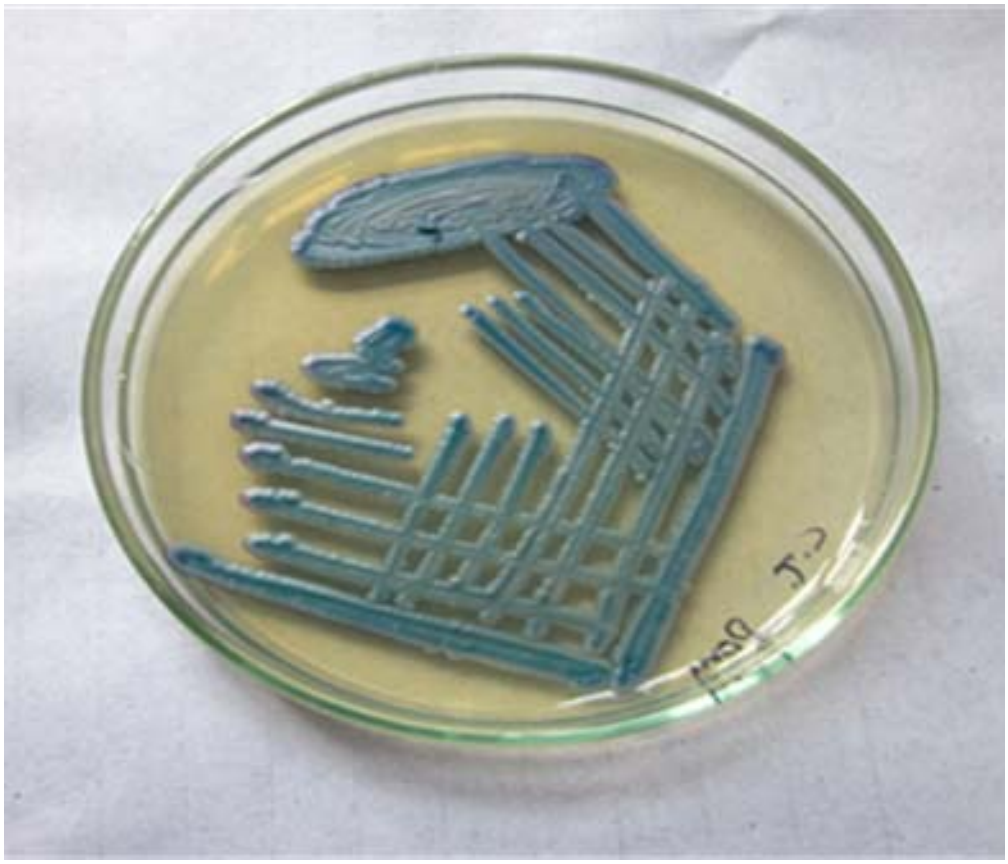
MICROSCOPY



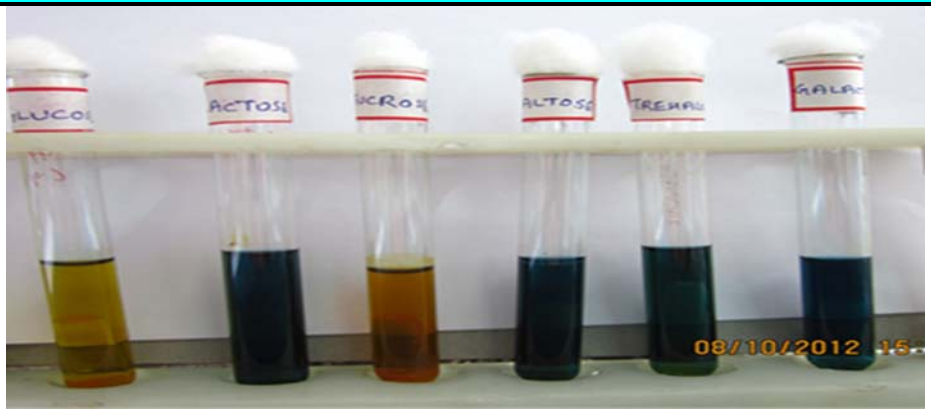
GERM TUBE TEST- NEGATIVE



CHROM AGAR-C.TROPICALIS

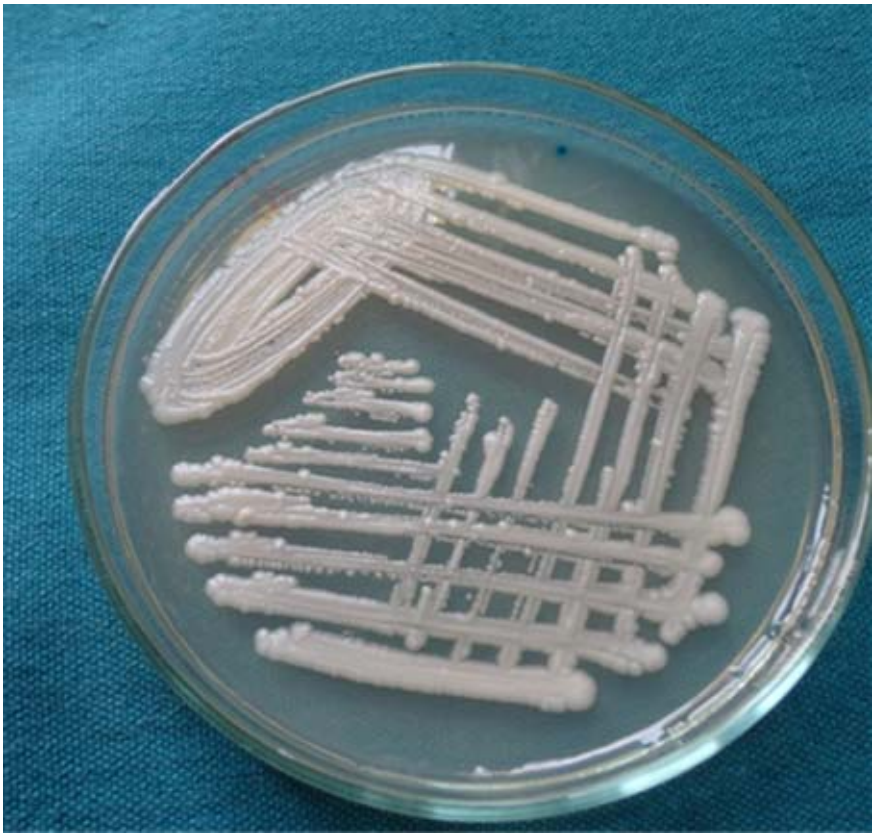


SUGAR FERMENTATION

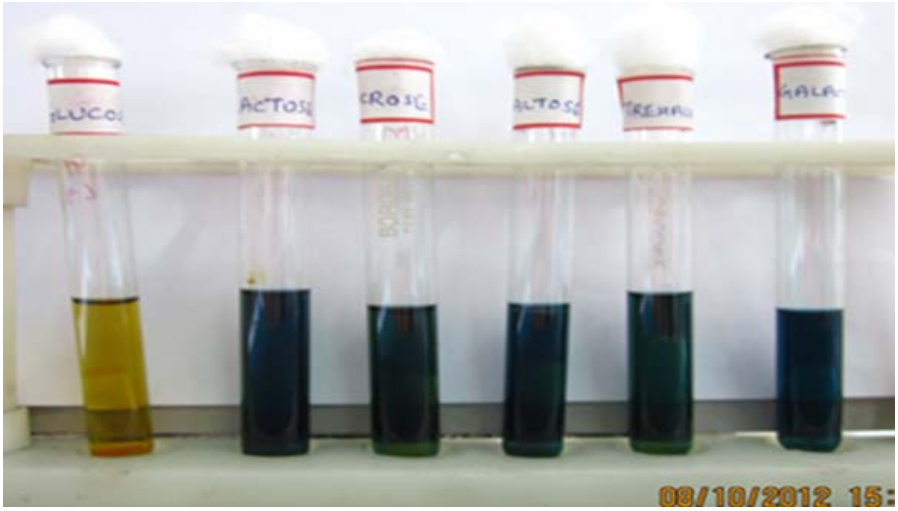


Glucose & Sucrose-Fermented

CHROM AGAR-C.PARAPSILOSIS

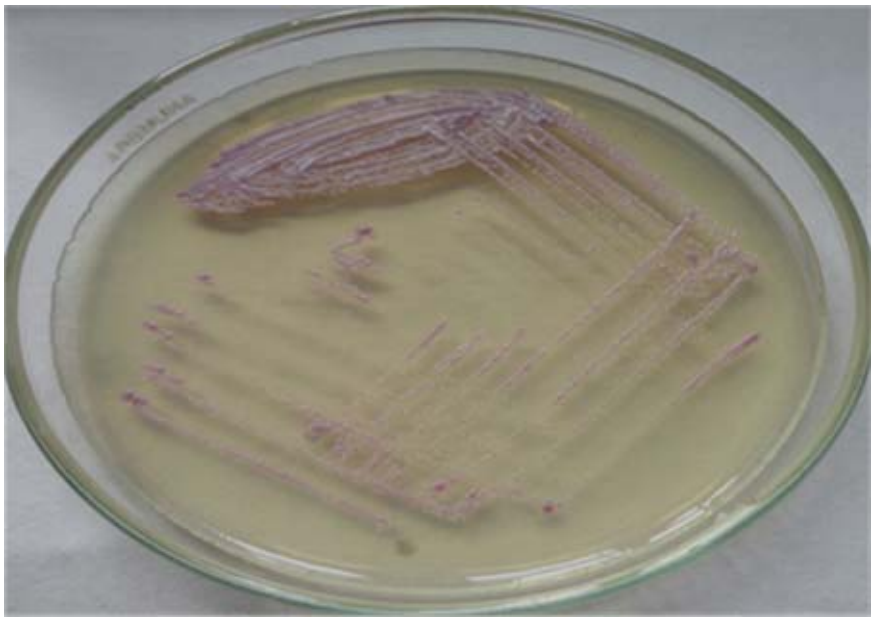


SUGAR FERMENTATION

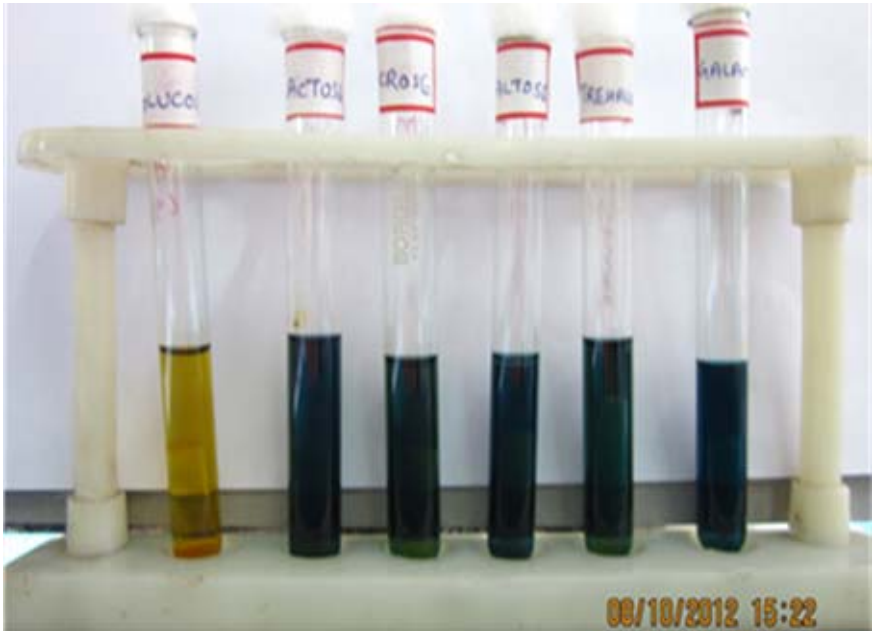


Glucose Fermented

CHROM AGAR-C.GLABRATA

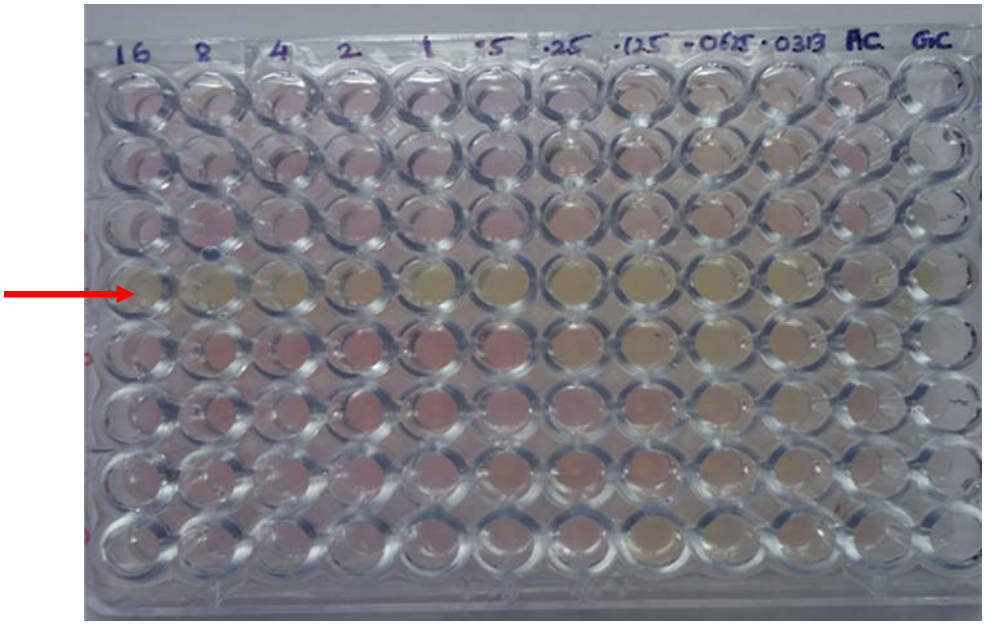


SUGAR FERMENTATION



Glucose Fermented

MIC OF ITRACONAZOLE - DERMATOPHYTE



Arrows shows Resisstant Strain- T.rubrum

MIC OF TERBINAFINE - DERMATOPHYTE

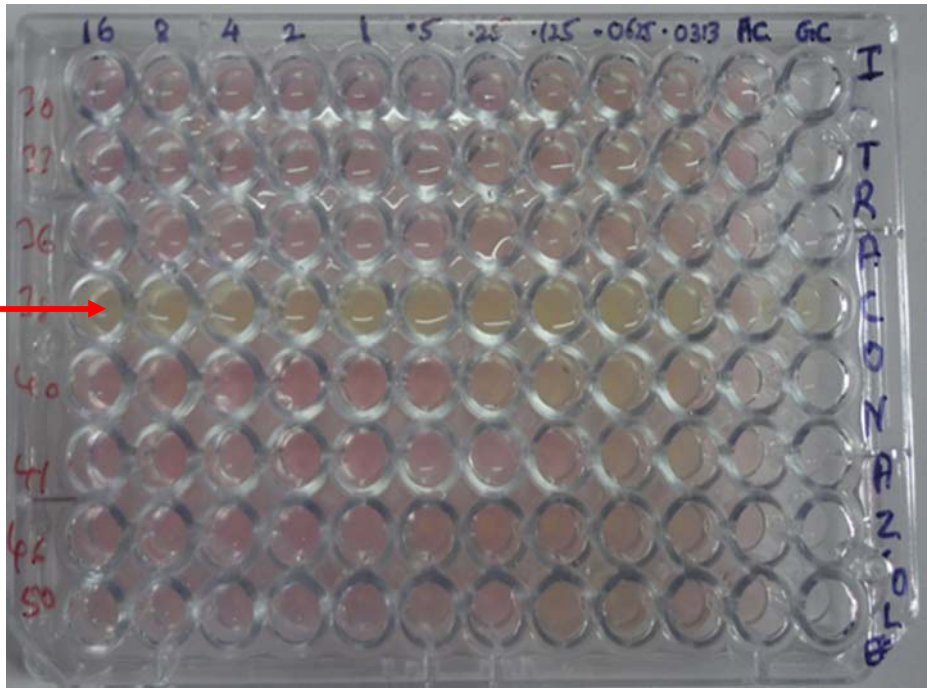


Image-19MIC OF AMPHOTERICIN B - NON DERMATOPHYTE MOULDS

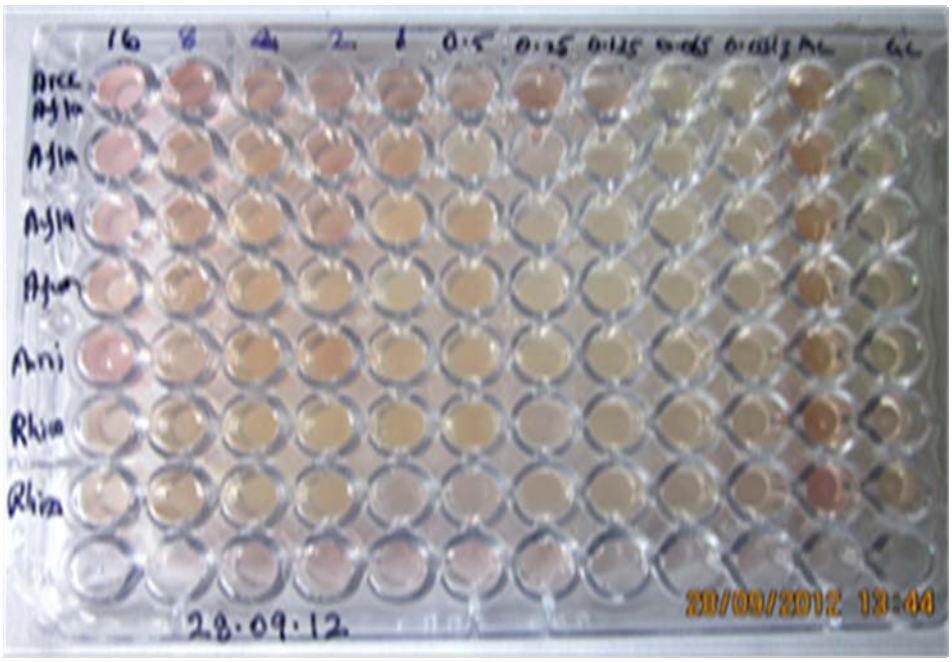
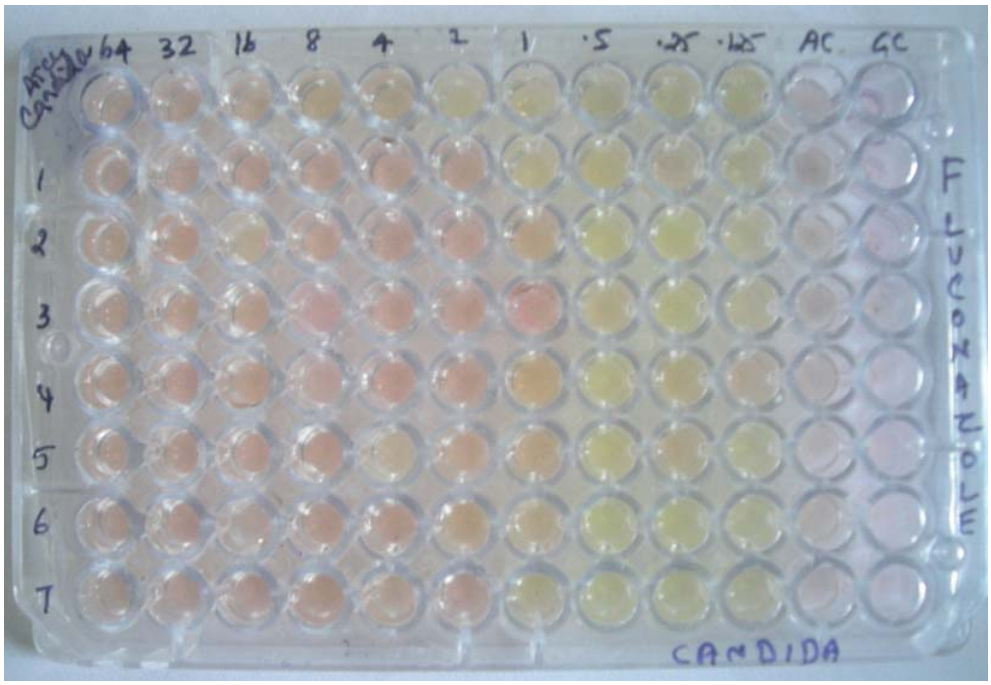


Image-20 MIC OF FLUCONAZOLE-CANDIDA SPECIES



INSTITUTIONAL ETHICS COMMITTEE
MADRAS MEDICAL COLLEGE, CHENNAI -3

Telephone No : 04425305301
Fax : 04425363970

CERTIFICATE OF APPROVAL

To
Dr. P. Ponnammal
PG in MD Microbiology
Madras Medical College, Chennai-3

Dear Dr. P. Ponnammal

The Institutional Ethics Committee of Madras Medical College reviewed and discussed your application for approval of the proposal entitled " A study on prevalence of superficial mycosis and its anti- fungal susceptibility pattern in diabetic patients in a tertiary care hospital " No. 20092011

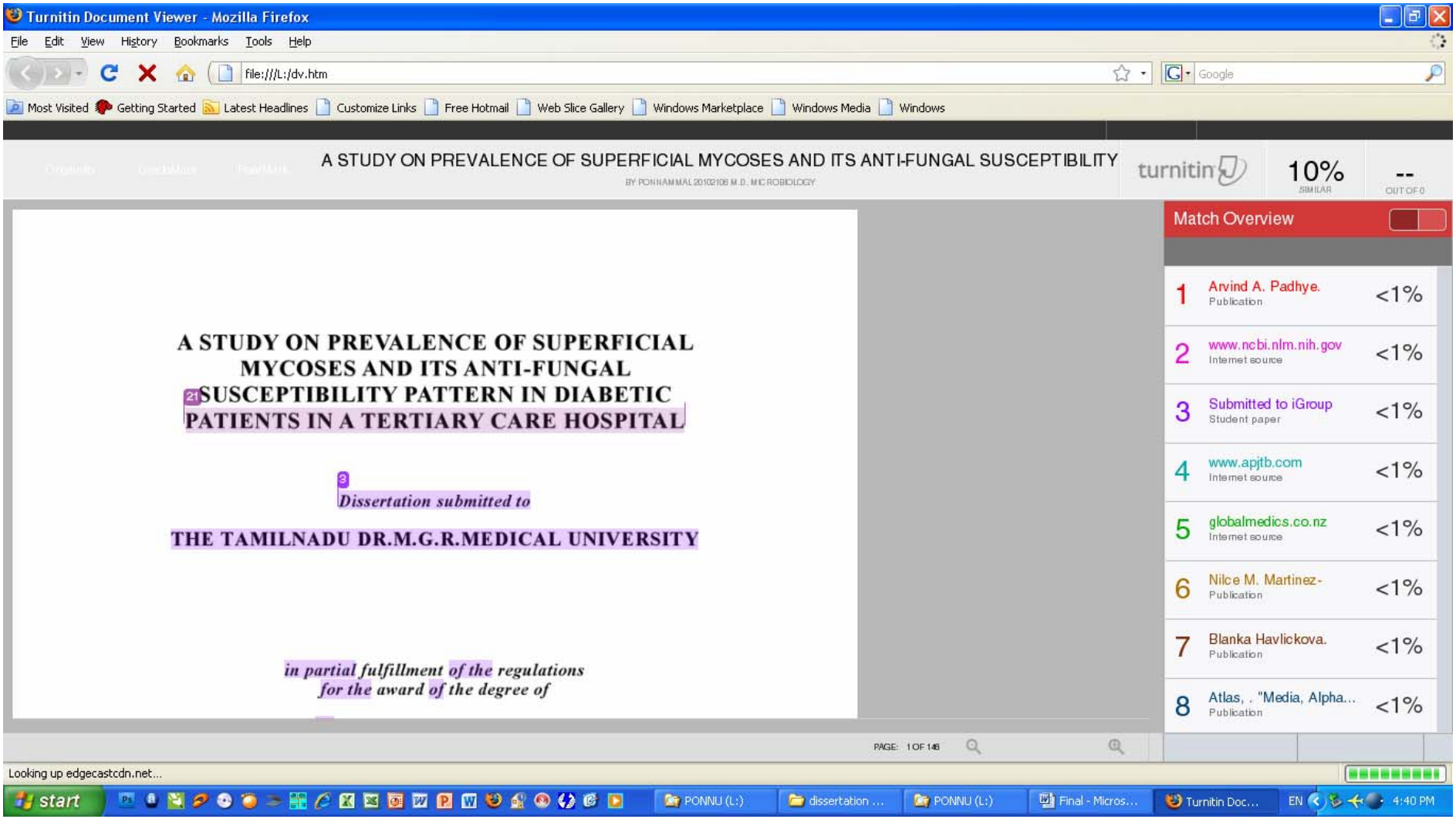
The following members of Ethics Committee were present in the meeting held on 27.09.2011 conducted at Madras Medical College, Chennai –3

1. Dr. S.K. Rajan MD	-- Chairperson
2. Dr. V. Kanagasabai MD Dean, Madras Medical College, Chennai -3	-- Deputy Chairman
3. Prof. R. Sundaram MD Vice Principal, Madras Medical College, Chennai -3	-- Member Secretary
4. Prof. R. Nandhini MD Director , Inst. of Pharmacology, MMC , Ch-3	-- Member
5. Prof. Pregna B. Dolia MD Director , Inst. of Biochemistry, M M C, Ch-3	-- Member
6. Thiru . Ulaganathan Administrative Officer, M M C, Ch-3	-- Layperson
7. Thiru. S. Govindasamy BA BL	-- Lawyer
8. Tmt. Arnold Saulina .MA., MSW	-- Social Scientist

We approve the Proposal to be conducted in its presented from

Sd/ Chairman & Other Members

The Institutional Ethics Committee expects to be informed about the progress of the study, any SAE occurring in the course of the study , any changes in the protocol and patient information / informed consent and asks to be provided a copy of the final report.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BY PONNAMMAL 20102106 M.D. MICROBIOLOGY
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